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Transport and Burial as a Cause of Whitefish (*Coregonus* sp.) Egg Mortality in a Eutrophic Lake

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Predictions based on the Shields diagram and confirmed by experiments conducted in eutrophic Lake Sempach imply that bottom currents associated with winter storm events are responsible for the simultaneous transport of coregonid eggs and fine silt and clay (grain size ≤ 10 μm) from the spawning grounds into deeper lake regions. The critical shear stress required to initiate egg transport is estimated to lie in the range 0.02–0.04 $N \cdot m^{-2}$, corresponding to mean current speeds of 10–15 $cm \cdot s^{-1}$ at a reference height of 0.5 m above the sediment surface. On settling out, egg burial is likely. This will increase egg mortality not only by physically hindering oxygen transport to the egg, but also, in POC-rich eutrophic lake sediment, by relocating the egg in a zone of steep oxygen gradients and low mean oxygen concentrations. Microelectrode measurements and computation of the thickness of the oxygen diffusive boundary layer over the sediment reveal that even eggs that escape interment are likely to be subjected to ambient oxygen concentrations insufficient for development to hatching. It is suggested that transport and burial may in general be important factors determining coregonid egg mortality in eutrophic lakes.

Des prévisions fondées sur le diagramme de Shields et confirmées par les expériences effectuées dans le lac eutrophe Sempach impliquent que les courants de fond associés aux tempêtes hivernales sont responsables du transport simultané d'oeufs de corégonidés, de limon et d'argile fins (taille des grains ≤ 10 μm), à partir des aires de frai jusqu'aux zones plus profondes du lac. On estime que la contrainte de cisaillement critique nécessaire pour déclencher le transport des oeufs se situe dans l'intervalle 0,02–0,04 $N \cdot m^{-2}$, ce qui correspond à des vitesses moyennes de courant de 10–15 $cm \cdot s^{-1}$ prises à une hauteur de référence de 0,5 m au-dessus de la surface des sédiments. Lors du dépôt des matériaux, il y a probablement enfouissement des oeufs. Cela augmentera le taux de mortalité des oeufs, non seulement en empêchant physiquement le transport d'oxygène jusqu'aux oeufs, mais également, dans les sédiments de lacs eutrophes riches en COP, en relocalisant l'oeuf dans une zone de forts gradients d'oxygène et de faible concentration moyenne d'oxygène. Des mesures par microélectrode et le calcul de l'épaisseur de la couche limite de diffusion de l'oxygène au-dessus des sédiments montrent que même les oeufs qui échappent à l'enfouissement seront peut-être exposés à des concentrations ambiantes d'oxygène insuffisantes pour qu'ils puissent évoluer vers le frai. On pense donc que le transport et l'enfouissement peuvent généralement représenter des facteurs importants lorsqu'on veut déterminer le taux de mortalité des oeufs de corégonidés dans les lacs eutrophes.

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Eutrophication is a major factor influencing the survival of whitefish and vendace (*Coregonus* sp.) populations in lakes (e.g. Nümann 1970; Hartmann 1977). The mechanisms by which eutrophication may lead to low coregonid population densities are, however, largely unknown. This paper is concerned with the influence of some physical and sedimentological factors on coregonid egg mortality in a eutrophic milieu.

Coregonids are broadcast spawners, spawning mostly in late autumn or winter when water temperature drops. Their eggs remain on the spawning grounds until hatching in spring and are therefore exposed for a long time to numerous environmental factors that can adversely influence their development and survival (Lahti et al. 1979). In addition to predation by fish and invertebrate fauna and to fungal attack (Mikkola et al. 1979; Schwank 1987), physical and chemical conditions at the sediment–water interface may also be responsible for the high egg mortality observed in eutrophic lakes. Thus, egg mortality tends to be highest on fine, muddy sediments and lowest on coarse, sandy substrates (Mikkola et al. 1976; Shemeikka et al. 1978). Fine sediment containing high proportions of organic material has a particularly detrimental effect on coregonid egg survival (Wilkońska and Żuromska 1982), presumably because of the high rates of oxygen depletion associated with such sediment (Lahti et al. 1979; Carlton et al. 1989).

Lake Sempach (surface area 14.5 km^2, maximum depth 87 m), situated in north-central Switzerland, has been highly eutrophic since about 1965 (Stadelmann 1980; Gächter et al.

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1983), with spring total phosphorus concentrations of 150–200 mg $P \cdot m^{-3}$ in the 1980s (Stadelmann 1988). It has a large whitefish population. To maintain the viability of a small-scale commercial fishing industry, the lake is stocked annually with hatchery-reared whitefish larvae. In order to increase ambient oxygen concentrations in the hypolimnion and reduce dissolution of bound phosphorus from the sediments, a lake restoration programme, including oxygenation in summer and artificial circulation in winter, was begun in 1984 (Gächter 1987; Stadelman 1988). Since one cause of impaired embryogenesis is commonly thought to be low hypolimnetic oxygen concentrations, it might be expected that this type of internal restoration measure might improve the viability of naturally spawned eggs. However, in a recent study of whitefish spawn in the littoral region of Lake Sempach, Ventling-Schwank and Müller (1991) demonstrated that over three successive spawning seasons (1987–90), no naturally spawned eggs were able to survive to hatching. Using a sled dredge and a diver-operated suction sampler to collect eggs from the lake bottom, they demonstrated that egg densities decreased drastically with time. Two weeks before the expected date of hatching, in spite of apparently favourable oxygen conditions (>7 mg $O_2 \cdot L^{-1}$ measured a few centimetres above the sediment surface), they were able to locate no viable eggs whatsoever. Survival of the Lake Sempach whitefish population is therefore entirely dependent on the stocking programme. Similar situations in other highly eutrophic Swiss lakes have been described by Müller (1992), who suggested that impaired embryogenesis may be caused by oxygen concentrations directly at the sediment–water interface being much lower than in the overlying bulk hypolimnetic water. The decline in egg numbers in the littoral zone of Lake Sempach observed by Ventling-Schwank and Müller (1991) led them to suggest that wind-induced currents may cause repeated translocation of spawn to deeper sites (cf. Büttiker 1986) with unfavourable oxygen conditions, possibly coupled with egg burial under disturbed sediments. The aim of the research described here was twofold: firstly, to formulate a transport and burial hypothesis and to test it by investigating egg and sediment transport in situ on the spawning grounds, and secondly, to examine the oxygen conditions prevailing in the microenvironment in which the eggs are located, i.e., directly at the sediment–water interface, using a microprobe. Although highly relevant to fish egg development, the oxygen conditions at the sediment–water interface have never previously been studied in this context.

Transport and Burial Hypothesis

Conditions in the physical environment to which fish eggs are exposed in lakes are governed to a large extent by the behaviour of water currents in the vicinity of the lake bottom. Sediment particles (and fish eggs) on the lake bottom are set in motion when the current-induced shear stress τ acting on them exceeds a certain magnitude. The physics of the boundary layer overlying the sediment is thus highly relevant to the problem under consideration here, since prediction of the conditions under which egg transport and/or burial can occur must be based on a suitable physical description of the mechanisms involved. Neglecting form drag due to topographic irregularities, the flow over a sediment surface in natural water bodies can often be appropriately described as a universally similar, neutrally buoyant boundary layer

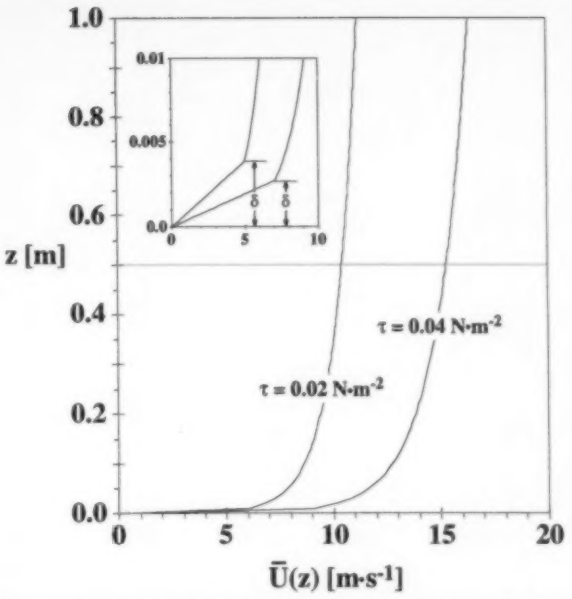

FIG. 1. Mean current velocity profiles over lake sediment. The profiles are linear (eq. (3)) within the viscous boundary layer (inset, $z \leq \delta$) and logarithmic (eq. (2)) within the turbulent boundary layer ($z \geq \delta$). The two profiles illustrated correspond to the lower and upper shear stresses τ (= 0.02 and 0.04 $N \cdot m^{-2}$, respectively) bounding the critical region within which it is predicted that the transport of coregonid eggs will be initiated. Current velocities were measured at a reference height of 0.5 m (dotted line) during the egg dispersion experiment.

flow over a smooth wall (e.g., Chriss and Caldwell 1984). In this case, for roughness Reynolds numbers $Re \leqslant 5$ (Yalin 1977), a viscous sublayer of thickness δ, in which molecular processes dominate, overlies the sediment, separating it from a turbulent flow regime extending into the bulk fluid (Fig. 1, inset). Fish eggs lying on the sediment surface will be situated largely within the viscous sublayer. Within this layer, the velocity profile is linear and the shear stress τ acting on the sediment surface is given by

$$(1)\quad \tau = \rho\nu \frac{\partial \bar{U}(z)}{\partial z}$$

where ρ = density of water $[ML^{-3}]$, ν = kinematic viscosity of water $[L^2T^{-1}]$, z = distance perpendicular to sediment surface $[L]$, and $\bar{U}(z)$ = mean current velocity at distance z $[LT^{-1}]$. Integrating eq. (1) and setting $\bar{U}(z) = 0$ at the sediment surface ($z = 0$) yields the expression for the linear velocity profile within the viscous sublayer:

$$(2)\quad \bar{U}(z) = \frac{\tau z}{\rho\nu} \qquad \text{for } z \leq \delta.$$

Within the turbulent layer overlying the viscous sublayer, the velocity profile is logarithmic. Continuity of the velocity profile over the two layers requires that the velocity profile in the turbulent layer be given (in nondimensional form) by

$$(3)\quad \frac{\bar{U}(z)}{u^*} = \frac{1}{\kappa} \ln \frac{z}{\delta} + \frac{u^* \delta}{\nu} \qquad \text{for } z \geq \delta$$

where $u^* = (\tau/\rho)^{1/2}$ is the friction velocity and κ (≈0.4) is the

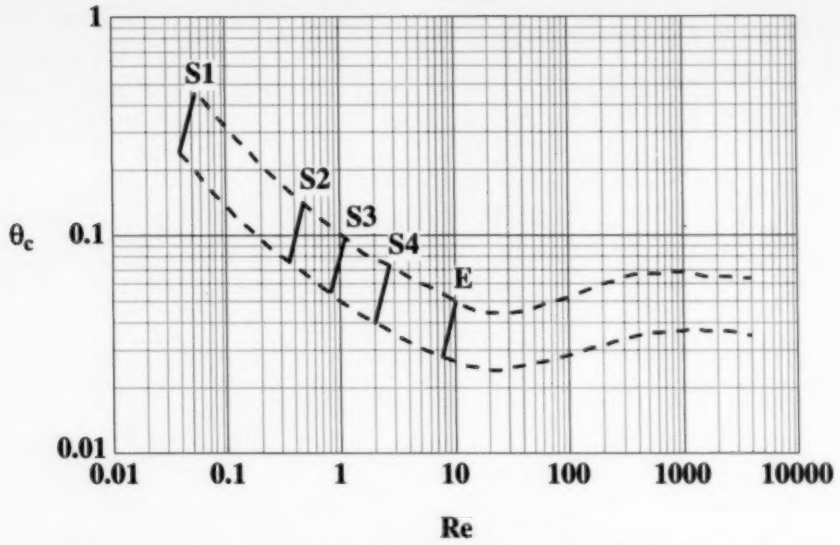

FIG. 2. Shields diagram as modified by Miller et al. (1977). The dashed lines represent the upper and lower limits of the critical nondimensional shear stress θ_c (eq. (4)) for the initiation of motion of noncohesive homogeneous sediment as a function of *Re* (eq. (5)). The critical region for sediment motion lies between the two curves. The straight solid lines represent the critical region for the initiation of transport of sediment of density 2650 kg·m^{-3} and grain diameter 10 (S1), 63 (S2), 125 (S3), and 250 μm (S4) and for the initiation of transport of coregonid eggs of density 1034 kg·m^{-3} and diameter 2.5 mm (E).

TABLE 1. Typical magnitudes of critical shear stress (τ_c), critical friction velocity (u_c^*), thickness of the viscous sublayer (δ), and mean current velocity at 0.5 m above the sediment ($\bar{U}_c$(0.5 m)) at the threshold of motion for a homogeneous sediment of grain diameter d at a water temperature of 4.5°C, calculated from the Shields diagram as modified by Miller et al. (1977) (Fig. 2).

d (μm)	τ_c (N·m^{-2})	u_c^* (cm·s^{-1})	δ (mm)	$\bar{U}_c$(0.5 m) (cm·s^{-1})
Homogeneous sediment (density = 2650 kg·m^{-3})				
10	0.04–0.07	0.6–0.9	2.8–2.0	15–21
63	0.08–0.14	0.9–1.2	1.9–1.5	21–30
125	0.11–0.20	1.1–1.4	1.6–1.2	26–36
250	0.16–0.29	1.3–1.7	1.4–1.0	32–44
Whitefish eggs (density = 1034 kg·m^{-3})				
2000	0.02–0.04	0.4–0.6	3.9–2.9	10–14
2500	0.02–0.04	0.5–0.6	3.5–2.6	11–15

von Kármán constant. Universal similarity requires the constancy of $u^*\delta/\nu$, and laboratory experiments imply a value of 11.6 (according to Yalin 1977) or 11.1 (according to Chriss and Caldwell 1984) for this term. Assuming universal similarity to apply, a knowledge of u^* and ν thus allows determination of δ, and hence of the form of the function $\bar{U}(z)$ over both layers (Fig. 1).

Based on experimental data from various sources, Shields (1936) expressed the critical nondimensional shear stress θ_c necessary to initiate motion of noncohesive homogeneous sediment as a function of *Re* in the so-called Shields diagram (Fig. 2). This critical nondimensional shear stress is given by

$$(4)\quad \theta_c = \frac{\tau_c}{g(\rho_s - \rho)d},$$

and the roughness Reynolds number at the critical shear stress by

$$(5)\quad Re = \frac{u_c^* d}{\nu}$$

where τ_c = critical shear stress [$ML^{-1}T^{-2}$], u_c^* = critical friction velocity [LT^{-1}], g = acceleration of gravity (=9.81 m·s^{-2}), ρ_s = density of sediment grains [ML^{-3}], and d = sediment grain size [L]. Assuming water temperatures of 4–5°C at the spawning grounds during most of the winter, $\rho \approx 1000$ kg·m^{-3} and $\nu \approx 1.54 \times 10^{-6}$ m^2·s^{-1} (Weast 1983). Making the usual assumption (e.g., Vanoni 1977) that the inorganic sediment grains have the density of quartz (i.e., $\rho_s \approx 2650$ kg·m^{-3}), the dependence of the critical shear stress τ_c on grain size d can be computed from the Shields diagram as revised and extended by Miller et al. (1977) to cover *Re* down to about 0.05 (Fig. 2). Although the critical shear stress τ_c is actually the parameter most relevant to sediment transport, it is current velocity, and not shear stress, that is generally measured directly. Prediction of the critical mean current velocity profile $\bar{U}_c(z)$ corresponding to a critical shear stress τ_c is accomplished by applying eq. (3) with $\delta = 11.1\ \nu/u_c^*$ (Fig. 1). In the experimental work described below, current speeds were measured at a height $z = 0.5$ m above the sediment surface, which will henceforth be used as a reference height.

An idea of the magnitudes of the critical shear stress τ_c and other related critical quantities for some representative grain

sizes corresponding to common sediment sieve mesh sizes is given in Table 1. Shear stresses of at least 0.04, 0.08, and 0.11 $N \cdot m^{-2}$ would be required in order to set homogeneous sediment of grain size 10, 63, and 125 μm, respectively, in motion. The corresponding critical mean current velocities (Table 1) are similar to those predicted by the empirical equation suggested by Miller et al. (1977) for the critical velocities required to initiate transport of quartz sediment in water at 20°C. Burial of fish eggs under inorganic silt coarser than 10 μm is thus unlikely unless the silt has first been set in motion by shear stresses exceeding 0.04 $N \cdot m^{-2}$ (associated with mean current velocities exceeding 15 $cm \cdot s^{-1}$ at 0.5 m above the sediment surface). The resuspension and subsequent settling out of inorganic silt particles finer than 10 μm may, however, be responsible for the burial of fish eggs at lower shear stresses. Whether this occurs or not depends at least in part on the critical shear stress required to set the eggs themselves in motion, since only static eggs will be subject to burial.

Although most frequently employed in the prediction of critical shear stresses for the initiation of motion of inorganic sediment with a density of 2650 $kg \cdot m^{-3}$, the validity of the Shields diagram is not restricted to material of this density, and in fact rests partly on experiments conducted on amber, which has a density of only 1060 $kg \cdot m^{-3}$ (Shields 1936). Thus, use of the same approach to determine a critical transport velocity for fish spawn suggests itself. Due to the degree of inhomogeneity involved, the Shields diagram cannot always be employed to determine a critical shear stress for fish spawn: critical shear stress values for individual eggs, for instance (or for individual sediment grains, for that matter), cannot be estimated in this way. However, eggs laid in patches can be treated as homogeneous sediment, possibly yielding even better estimates of critical shear stress than in the case of natural inorganic sediment, which by its very nature is inhomogeneous.

The densities of a representative sample of eggs were determined by allowing the eggs to settle or float in three settling columns containing NaCl solutions of densities 1031, 1034, and 1037 $kg \cdot m^{-3}$ (densities calculated according to Weast 1983, p. D-238). A total of 39 eggs in an early developmental stage (blastoderm enveloping yolk) and 36 eggs in a later stage (pigmented eyes) were tested. Of these, 79% of the former and 69% of the latter lay within the density range 1031–1037 $kg \cdot m^{-3}$. Thus, a mean density of 1034 $kg \cdot m^{-3}$ can be assumed with little error. However, in the field, fine sediment may adhere to the surface of the eggs, increasing their effective density, and thus theoretical critical velocity determinations may represent a lower limit to the actual critical velocities required to initiate motion. The egg diameter varied between 2.0 and 2.5 mm, but from Table 1 it can be seen that the critical parameters are insensitive to egg size. The estimated critical shear stress τ_c for egg transport lies between 0.02 and 0.04 $N \cdot m^{-2}$, comparable with that required for the initiation of motion of fine inorganic silt ($d \leq 10$ μm).

The predictions of the model can thus be summarized as follows. Whitefish eggs are expected to remain static if the shear stress does not exceed 0.02 $N \cdot m^{-2}$ and to be in motion if it exceeds 0.04 $N \cdot m^{-2}$. A shear stress of up to 0.02 $N \cdot m^{-2}$ should suffice to cause resuspension of very fine nonconsolidated inorganic silt and clay particles, which may cover static eggs upon settling out. Eggs in motion when the shear stress lies within the range 0.04–0.07 $N \cdot m^{-2}$ will be accompanied by coarser silt particles, which will, however, not normally exceed 10 μm in diameter. A much higher shear stress is required to set sediment coarser than this in motion.

Experiment

Egg Dispersion Experiment

To test the above predictions, an in situ egg dispersion experiment involving the observation of the translocation and burial of whitefish eggs in conjunction with measurements of wind speed and current speed was conducted in Lake Sempach. Whitefish spawn from the beginning of December to the beginning of January all over the littoral zone of this lake, showing no preference for particular spawning grounds. Hatching should be about 2 mo later. The spawning site chosen for the experiment, which was already well known from the previous egg survival study (Ventling-Schwank and Müller 1991), was located on a gently sloping area within the littoral zone. The experiment employed a concentric arrangement of fences (Fig. 3) situated at a depth of 3–9 m. An inner circle of eight X-shaped 1-mm-mesh nylon gauze fences (radius 2 m) was surrounded by an outer circle of 12 similar fences (radius 5 m). Three V-shaped fences 10 m southwest of the centre of the two concentric circles completed the installation.

On 16 January 1990, 3925 eggs dyed with cresyl violet were deposited within a small area (0.5 m^2) at the centre of the fence arrangement. The location of the eggs and the condition of the experimental installation were checked regularly; eggs that had been transported away from their original location and had become trapped at the gauze fences were collected using a specially developed diver-operated suction sampler (Ventling-Schwank 1992) for subsequent counting in the laboratory. A similar experiment was initiated on 24 January 1990 with the same number of eggs, marked this time with toluidine blue to avoid confusion. Preliminary experiments had confirmed that neither of the histological dyes had any adverse effects on egg survival.

During the experiment, 5-min-averaged current velocities were measured using a Neil Brown acoustic current meter (EG & G Marine Instruments, Cataumet, Mass.) located on a tripod 0.5 m above the lake bottom and 6 m under the surface. Wind speed data were available from two automatic meteorological stations belonging to the Swiss Meteorological Office (Buchs-Suhr, 26 km to the northwest, and Lucerne, 15 km to the southeast). Hourly mean wind speeds measured at these two stations are correlated significantly (for December and January data from December 1984 to January 1992, four one-hourly means per day: $n = 1984$, $r = 0.41$, $p < 0.01$); the mean of the wind speeds measured at these two stations (W) can thus be taken to be a good relative indicator of the wind speed conditions prevailing at Lake Sempach.

Between 11 January and 23 January 1990 the hourly mean wind speed indicator (W: Fig. 4a) and the 5-min mean measured current speed 0.5 m over the spawning ground ($\bar{U}$(0.5 m): Fig. 4b) rarely exceeded 2 and 5.3 $cm \cdot s^{-1}$, respectively. Therefore, from eq. (3), the current-induced shear stress τ in the bottom boundary layer did not exceed 0.006 $N \cdot m^{-2}$. After deposition of the first batch of 3925 marked (violet) eggs at the centre of the arrangement of concentric fences on 16 January 1990, regular dives were carried out to inspect the position and condition of the eggs.

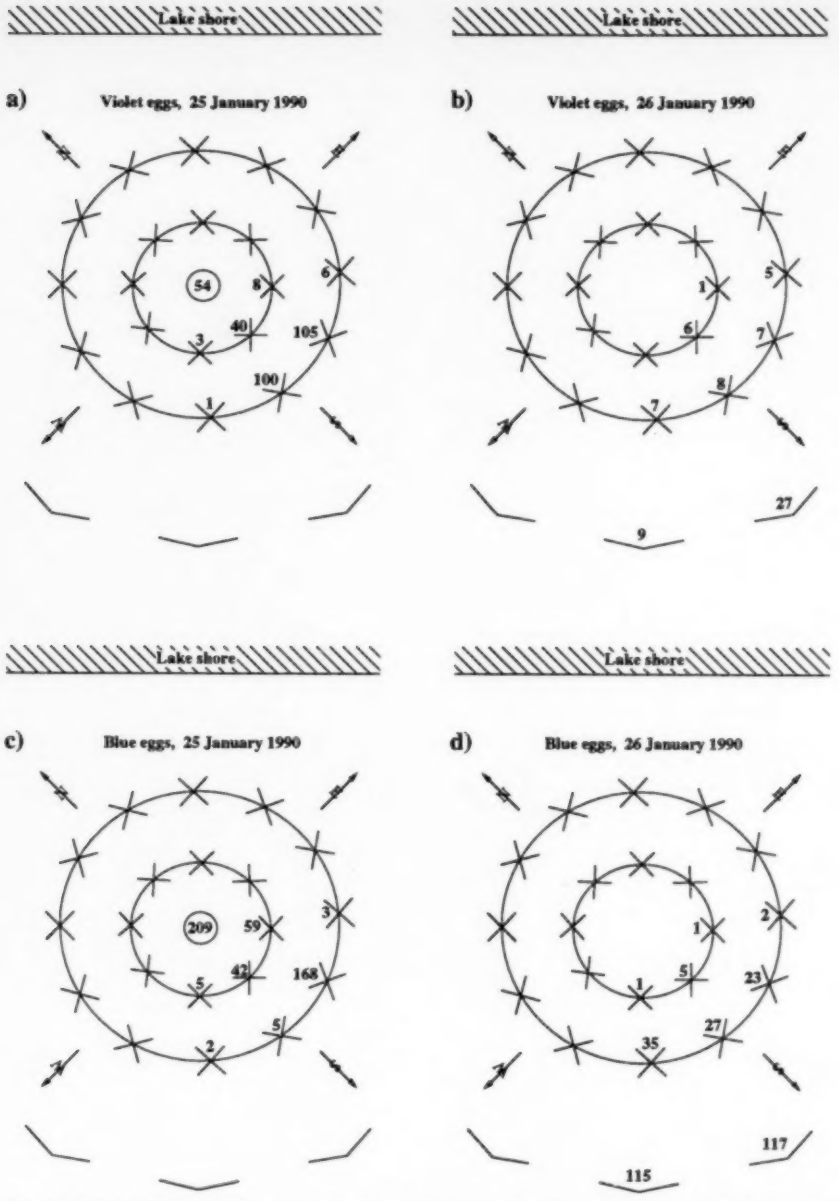

FIG. 3. Schematic plan of the concentric fence arrangement deployed on the whitefish spawning grounds during the egg dispersion experiment, showing the location of marked eggs collected during the experiment. Total number of eggs deposited at centre of installation: 3925 (marked with cresyl violet) on 16 January 1990 and 3925 (marked with toluidine blue) on 24 January 1990. (a) Violet eggs (317) collected on 25 January 1990. (b) Violet eggs (70) collected on 26 January 1990. (c) Blue eggs (493) collected on 25 January 1990. (d) Blue eggs (326) collected on 26 January 1990.

No translocation of any of the marked eggs was observed up to and including 23 January 1990. On 24 January 1990, a heavy storm began which lasted 3 d. High westerly winds (Fig. 4a) resulted in correspondingly high current speeds (Fig. 4b). Peak values of 6.9 $m \cdot s^{-1}$ for the hourly mean wind speed indicator and 17.7 $cm \cdot s^{-1}$ for the 5-min mean current speed (corresponding to $\tau = 0.05$ $N \cdot m^{-2}$) were recorded during the storm. The hourly mean current speed during the storm was highly correlated with the hourly mean wind speed indicator ($n = 153$, $r = 0.81$, $p < 0.01$).

Qualitative conditions under water during the storm were noted on the dive carried out on 24 January. Down to a depth of 4 m, a fluctuating current motion was apparent at the sediment surface which resulted in the formation of

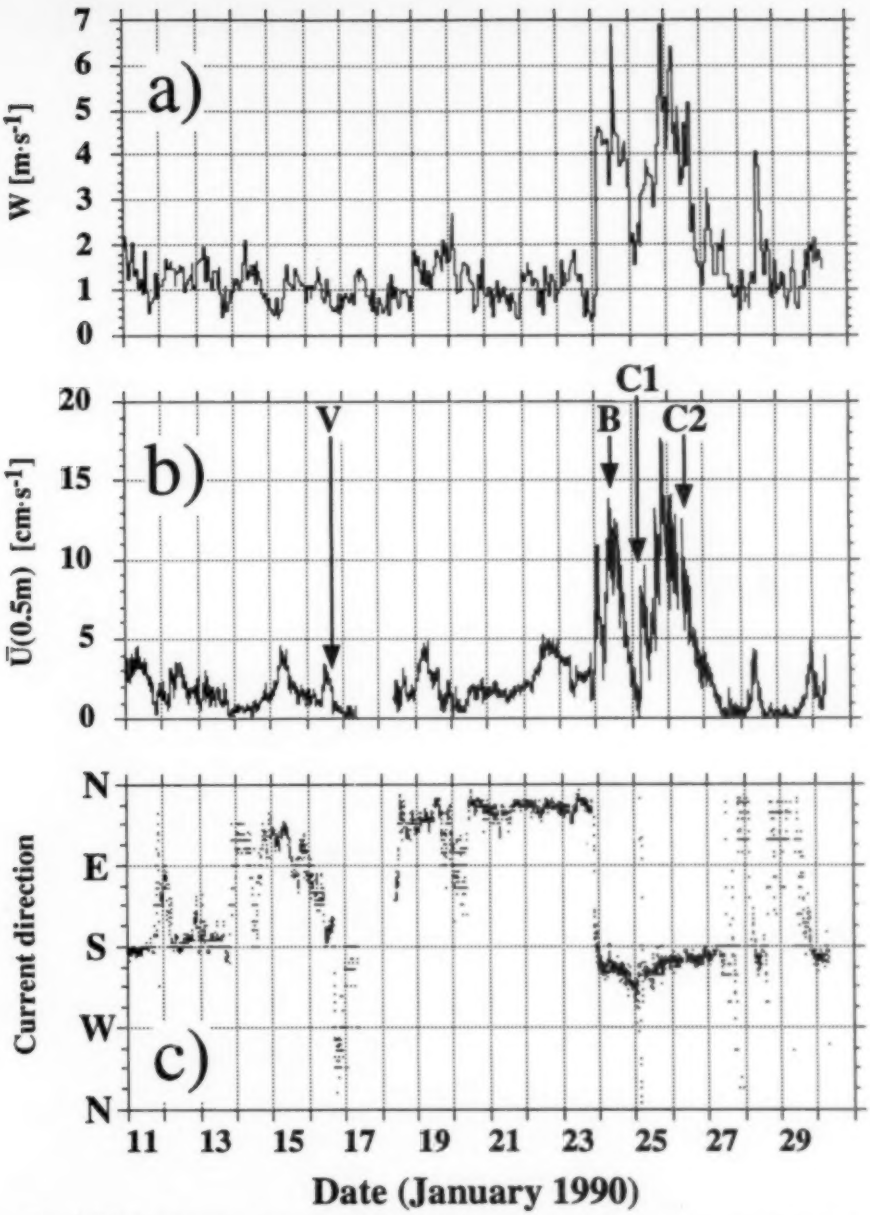

FIG. 4. Wind and current data measured during the egg dispersion experiment (11–30 January 1990). (a) Mean wind speed *W* (hourly means) measured at the Buchs-Suhr and Lucerne meteorological stations. (b) Current speed (5-min means) measured 0.5 m above the spawning ground and 6 m below the water surface at the location of the experimental arrangement of Fig. 3. The arrows denote the times at which the marked eggs were deposited (V: violet eggs; B: blue eggs) and collected (C1, C2). (c) Current directions corresponding to the current speeds of Fig. 4b.

ripples. The fluctuations were strongest close to the lake shore, decreasing in intensity with increasing depth, until at the depth at which the eggs had been deposited (6 m), no signs of any fluctuations were observable. Visibility was reduced from the usual 4–5 m down to 2 m by the presence of resuspended sediment. Marked (violet) whitefish eggs, along with large quantities of various other organic material (plant fibres, leaves, etc.), were observed to have been trapped at the concentric fences. For technical reasons, it was not possible to sample the violet eggs on 24 January; however, on that day we were able to deposit a second batch of 3925 marked eggs (blue) at the centre of the arrangement. On 25 January, 30 h after commencement of the storm, a total of 317 violet and 493 blue eggs were collected within

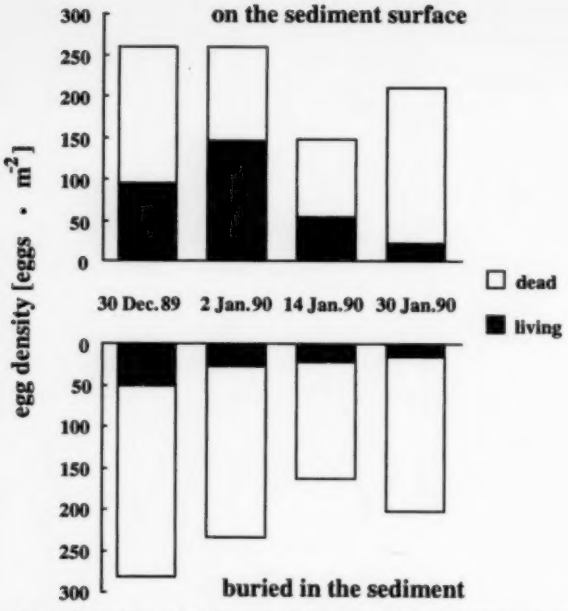

FIG. 5. Densities of living and dead coregonid eggs sampled on and in the Lake Sempach sediment on four different dates. Each column represents the mean of six separate samples.

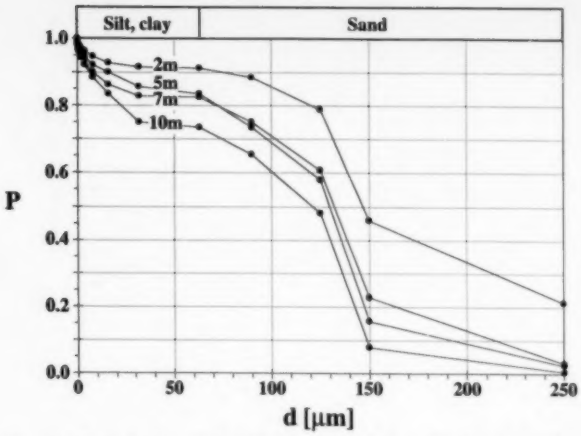

FIG. 6. Cumulative probability distributions of sediment grain size (equivalent spherical diameter d) in the uppermost centimetre of sediment sampled at 2, 5, 7, and 10 m depth in Lake Sempach in February and March 1990. P is the probability of d exceeding a given value.

5 m of the centre (Fig. 3). The eggs in the centre were partly covered with sediment. On 26 January the sampling was repeated, this time up to the fences situated 10 m southwest of the centre. During this second sampling, 70 violet and 326 blue eggs were found. In total, 9.9% of the violet eggs and 20.9% of the blue eggs were recovered.

During the entire period of the storm, the current was directed southward at an angle of about 45° away from the shore (Fig. 4c). Almost all the eggs recovered — from both batches and on both sampling dates — were situated within a sector extending from southwest to southeast (Fig. 3), implying that eggs were transported both parallel and perpendicular to the shore in the general direction of the current.

Over the period 11–23 January 1991, W rarely exceeded 2 m·s^{-1} whereas during the period 24–26 January 1991, the mean value of W was 3.8 m·s^{-1} and peak values as high as 6.9 m·s^{-1} were recorded. An analysis of the wind speed indicator W during the months of December and January from December 1984 to January 1992 revealed that W exceeded 2 m·s^{-1} during 25% of the time and exceeded 4 m·s^{-1} during 5% of the time. Wind conditions similar to those encountered during the course of the egg dispersion experiment are thus likely to be encountered every winter. It can therefore be assumed that the transport of eggs and sediment, as observed in January 1990, is also likely to occur every winter.

Egg Burial

As mentioned above, the storm on 24 January 1990 resulted in those eggs that had remained static being partly covered with sediment. The possibility of egg burial having occurred at other times during the spawning season, both before and after the storm, was investigated at a distance of 10–30 m from the experimental installation by sampling naturally spawned eggs and sediment using the above-mentioned suction sampler. Where eggs were visible, an aluminium frame of area 0.5 m^2 was placed on the sediment. All eggs visible on the surface of the sediment were collected, and then the uppermost 2 cm of sediment was removed with the suction sampler and transported to the laboratory, where all samples were preserved in Stockard solution (85 parts water, five parts 40% formalin, five parts glycerine, and five parts acetic acid) for later examination. Six samples taken on four different dates (30 December 1989 and 2, 14, and 30 January 1990) in depths of 3–9 m were examined for living and dead eggs. In all samples, approximately half the number of eggs collected (46% on average) were found buried in the sediment (Fig. 5). Pooling all samples, only 13% of the buried eggs were found to be living, as opposed to 36% of eggs lying on the surface. Burial in the sediment thus, not surprisingly, appears to have a detrimental effect on egg viability.

Sediment Characterization

The nature of the sediment upon which coregonid eggs lie is important for their chances of survival. The two sediment properties most relevant to the problem at hand are grain size distribution and particulate organic carbon (POC) content. These were determined quantitatively.

Samples of the uppermost centimetre of sediment at four different depths below the lake surface (2, 5, 7, and 10 m) obtained in the vicinity of the experimental installation during dives in February and March 1990 were divided into six fractions according to grain size (particle diameter d) by wet sieving using sieves of mesh sizes 63, 90, 125, 150, and 250 μm. The finest fraction ($d \leq 63$ μm) was subdivided into nine further fractions using an automated particle size analyser (Sedigraph 4600, Micromeritics Instruments Inc.). At all four depths, the sand fraction (grain diameter $d >$ 63 μm) dominates in the upper centimetre of sediment (Fig. 6). Medium sand (125 μm $< d \leq$ 150 μm) accounts for 35–40% of the sediment in all samples, while coarse sand (>250 μm) accounts for a significant proportion of the sediment only in the 2-m sample. The proportion of silt and

clay ($d \leq 63$ μm) increases with depth from 9% at 2 m to 27% at 10 m. Most of this fine fraction is silt (2 μm < $d \leq$ 63 μm); the clay fraction ($d \leq 2$ μm) did not exceed 6% of the total in any sample.

For the determination of POC concentrations, the sediment was dried and pulverised, and inorganic carbon (carbonate) was expelled as CO_2 by treating each 50-mg sediment sample with 25 mL of 0.5 M HCl. The samples were homogenised ultrasonically and filtered through GF/F glass fibre filters. After drying at room temperature, POC concentrations were determined using a Heraeus CHN analyser. Surface sediment POC concentrations were found to be practically constant from 2 to 10 m depth, ranging from 10.4 to 12.7 mg POC·g sediment^{-1}.

POC concentrations in deeper regions of the lake are considerably higher than this. Obenlühneschloss (1986), for instance, who took 69 cores in Lake Sempach in 1984–85, found POC concentrations in surface sediments to increase steadily with depth, exceeding 55 mg POC·g sediment^{-1} in the deepest parts of the lake, while Höhener (1990) found as much as 75 mg POC·g sediment^{-1} in the surface sediment of cores taken in May and November 1988 and January 1989 at the deepest point of the lake (87 m).

Oxygen Conditions

Information on oxygen conditions at the sediment–water interface was obtained from measurements made on two 12-cm-diameter cores taken on 23 November 1990 from the littoral zone of Lake Sempach at 8 and 15 m water depth. Oxygen concentrations were measured approximately 15 min after the cores were taken. Using a micromanipulator, the oxygen probe (Eschweiler, Kiel, Germany) was gradually lowered through the water until it penetrated the sediment. The water was not stirred either before or during measurement. Two-point linear calibration was carried out using a saturated oxygen solution and the oxygen concentration in the bulk water overlying the sediment, which was determined using the Winkler method.

Although the surface sediment from the core taken at 8 m depth was much lighter in colour than that from the core taken at 15 m depth and also contained a higher proportion of sand, the oxygen profiles from both cores were similar (Fig. 7). Whereas oxygen concentrations in the bulk overlying water exceeded 6 mg $O_2 \cdot L^{-1}$, a rapid decrease in the oxygen concentration occurred 1.5–2.5 mm above the sediment surface. At the sediment–water interface, concentrations of 3–4 mg $O_2 \cdot L^{-1}$ were measured, and within the sediment, the oxygen concentration fell to zero at 2.5 mm although the sediment was still light in colour, and thus presumably oxidised, down to about 5–10 cm below the surface. These results are qualitatively similar to recent results obtained with microelectrodes over and within coastal marine sediments (Jørgensen and Revsbech 1985; Gundersen and Jørgensen 1990) and saline pond sediments (Jørgensen and Des Marais 1990).

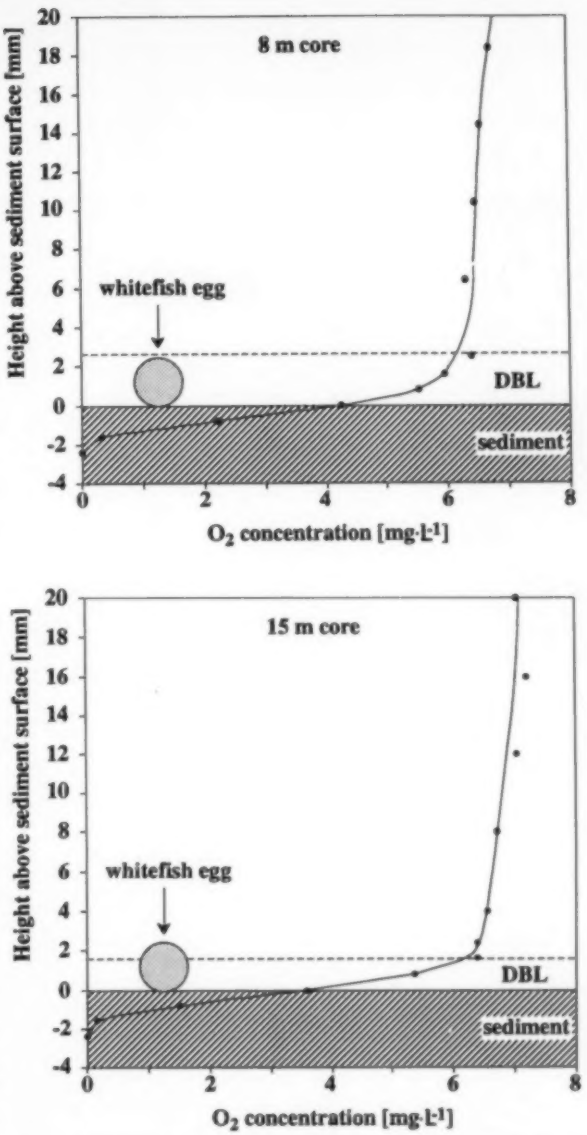

FIG. 7. Oxygen profiles measured with a microelectrode in the vicinity of the sediment–water interface in cores taken at depths of 8 and 15 m in Lake Sempach. "DBL" denotes the diffusive boundary layer for oxygen over the sediment. A whitefish egg of diameter 2.5 mm is included for comparison purposes.

Discussion

Egg and Sediment Transport: Comparison of Theory and Experiment

The results of the egg dispersion experiment support the predictions made on the basis of the Shields diagram, suggesting that this approach may be of general value in making predictions concerned with the transport and burial of fish spawn. No egg transport occurred during the early part of the egg dispersion experiment, implying that, as predicted, the current-induced shear stress was too low to set the eggs in motion. During the storm, $\bar{U}(0.5\text{ m})$ moved into the range 10 cm·s^{-1} $\leq \bar{U}(0.5\text{ m}) \leq$ 15 cm·s^{-1}, yielding shear stresses within the critical range 0.02 N·m^{-2} $\leq \tau \leq$ 0.04 N·m^{-2} predicted to initiate egg motion, and egg transport did indeed occur. The critical shear stress required to initiate motion of the eggs is predicted to be comparable with that required to initiate motion of the fine silt and clay fractions ($d \leq$

10 μm). Thus, at current velocities and shear stresses of the magnitude found associated with high-wind events, it is to be expected that coregonid eggs and fine inorganic sediment particles will be set in motion approximately simultaneously, increasing the probability of the eggs being buried after cessation of motion and subsequent settling of both eggs and sediment. The spectrum of grain sizes present in heterogeneous sediment will settle out at different rates: coarser grains will settle out before the eggs and finer grains after, tending to "sandwich" the eggs in the settled sediment. Apart from the partial burial of the eggs in the centre of the experimental installation during the storm, there is no in situ observational evidence for a direct cause-and-effect relationship between storm events and egg burial. However, the model strongly suggests that such a relationship does exist. Since coregonids are broadcast spawners and neither clean the spawning grounds nor bury their eggs themselves, and since the density of inorganic sediment (2650 $kg \cdot m^{-3}$) considerably exceeds that of the eggs (1034 $kg \cdot m^{-3}$), there is no other plausible explanation for the high proportion of eggs found within, as opposed to on, the sediment than burial as a result of sediment resuspension and subsequent settling. Each storm event is likely to result in an increase in the proportion of buried eggs, which may explain the decrease in egg densities with time found by Ventling-Schwank and Müller (1991).

Effect of Egg Translocation on Substrate Quality

Because of the so-called "funnel effect", eggs are more likely to be transported into deeper than into shallower water. Transport into deeper water in itself is unlikely to have a detrimental effect on egg survival, since coregonid eggs will survive at any depth if substrate, oxygen concentration, temperature, and pH are suitable (Lahti et al. 1979). However, both substrate quality and oxygen conditions deteriorate with increasing depth.

From Fig. 6 it is apparent that the average sediment grain size decreases with increasing depth below the lake surface. This results from three processes. Firstly, since the critical shear stress is related to the sediment grain size, a decrease in bottom current speed with depth (the largest current speeds are likely to be found in the littoral surface wave zone) will result in a depth dependence of the sediment grain size distribution. Secondly, even if current speed were independent of depth, repeated preferential transport of finer material over a sloping bottom will give rise to a gradual increase in the proportion of finer sediment with increasing depth because of the "funnel effect". Thirdly, the fact that the specific gravity of turbid lake water exceeds that of clear water will tend to reinforce the "funnel effect". Eggs translocated to deeper lake regions will therefore be deposited on, or within, sediment which is much finer than that of the spawning grounds.

In Lake Sempach, surface sediment POC concentrations increase by several hundred percent on proceeding from littoral to profundal regions. According to Wood (1964), organic material has almost the same density as water and tends to settle out in the deeper regions of lakes. Thus, most sediments in the profundal regions of eutrophic lakes tend to conserve carbon, since sedimentation rates there exceed decomposition rates (Kerr et al. 1973). In general, therefore, surface sediment POC concentrations increase with water depth. POC concentrations in sediment samples taken from between 2 and 10 m water depth in Lake Sempach were relatively low. Surprisingly, the oxygen gradients measured close to the sediment surface at these depths were quite steep, indicating that a large amount of oxygen is required for mineralisation processes. In shallow (littoral) regions of lakes, wave and current action ensure a constant supply of oxygen to the sediments, allowing rapid mineralisation to take place. In profoundal regions, this is no longer the case. With increasing depth, the increasing POC content of eutrophic lake sediment intensifies the sediment oxygen demand. The oxygen supply to the sediment from the overlying bulk water worsens with increasing depth, however, and is less likely to be able to meet the higher demand. Eggs translocated to deeper regions of eutrophic lakes will therefore be exposed to lower oxygen concentrations than those found at the spawning grounds.

Aquarium experiments by Mikkola et al. (1976) have demonstrated that coregonid egg mortality depends on substrate type. The proportion of coregonid eggs found by them to survive until hatching was 26% on sand, 16% on firm mud, and 0% on soft mud. Field and aquarium experiments by Wilkońska and Żuromska (1982) have also shown that the presence of fine, light colloidal particles in the sediment can result in coregonid egg mortality. According to Żuromska (1982), whitefish prefer spawning grounds that are hard, stony, and gravelly. This type of substrate, however, is rare away from the immediate vicinity of the lake shore or of inflowing streams. Eggs transported away from the spawning grounds will tend to be deposited on a substrate that is finer, richer in POC and has a higher oxygen demand than that of the spawning grounds. Thus, egg transport, whether or not followed by burial, is likely to be detrimental to egg survival. The oxygen conditions in the micro-environment to which the translocated eggs are exposed — either the uppermost few millimetres of sediment, if buried, or the lowermost few millimetres of water, if not — are therefore of paramount importance for egg survival.

Significance of the Oxygen Diffusive Boundary Layer for Egg Mortality

About half the eggs sampled in December 1989 and January 1990 were found buried in the sediment (Fig. 5). Burial under a layer of fine sediment reduces egg survival chances for two reasons. Firstly, a layer of sediment several millimetres thick can form a purely physical barrier hindering the transport of oxygen to the egg. Fudge and Bodaly (1984), for instance, showed in an experiment in Southern Indian Lake, Manitoba, that the sedimentation of sand and silt poor in organic matter (<4%) substantially reduced the survival chances of whitefish eggs. Secondly, the presence of organic matter in eutrophic lake sediment results in a steep gradient in the oxygen profile in the immediate vicinity of the sediment–water interface and in anoxic conditions a few millimetres below the interface (Fig. 7). Before the availability of microelectrodes, it was assumed that the penetration depth of oxygen in aquatic sediments was similar to the thickness of the oxidised layer (often light brown) (Carlton et al. 1989). Revsbech et al. (1980a, 1980b) showed, however, that anoxic conditions in surface sediment exhibiting a positive redox potential down to 10 cm begin only a few millimetres below the interface. Thus, a thin layer of sediment overlying a fish egg does not merely act as a physical barrier to the diffusion of oxygen from the water to the egg; it also

drastically increases the probability of the egg being situated in an environment of low or zero oxygen concentration, in which its survival chances are negligible. In fact, even eggs lying on the sediment surface are likely to experience unfavourable oxygen conditions, as the measurements over the Lake Sempach sediment show (Fig. 7). The oxygen concentration is low not only at the sediment surface, but also in the diffusive boundary layer (DBL) overlying the sediment surface (cf. Jørgensen and Revsbech 1985; Gundersen and Jørgensen 1990; Jørgensen and Des Marais 1990). Oxygen consumed during bacterial degradation of organic material in the sediment can only be replaced by molecular diffusion through the DBL. At low current speeds, a whitefish egg with a typical diameter of 2–2.5 mm lying on the sediment surface will be situated almost wholly within the DBL and will be subjected to its steep oxygen gradient (Fig. 7). The survival of the egg is thus dependent on the oxygen supply within the DBL and on the thickness of the DBL (although natural convection resulting from egg respiration may improve the oxygen supply somewhat: see O'Brien et al. 1978). In the DBL above the Lake Sempach sediment, oxygen concentrations ranged from 3.5 to 6 mg $O_2 \cdot L^{-1}$.

Little is known of the oxygen requirements of whitefish eggs. However, Brooke and Colby (1980) have shown that the mortality of *Coregonus artedii* eggs undergoes a drastic increase when oxygen concentrations are reduced to 4 mg $O_2 \cdot L^{-1}$ at 6–8°C or 2 mg $O_2 \cdot L^{-1}$ at 2–4°C. Such low oxygen concentrations can therefore be regarded as insufficient for development. Oxygen requirements during embryogenesis are, however, not constant, but increase continually. In the early stages of their development, salmonid eggs require a minimum ambient oxygen concentration of 1–3 mg $O_2 \cdot L^{-1}$. Immediately before hatching, however, they require 7–10 mg $O_2 \cdot L^{-1}$ (Hayes et al. 1951; Alderdice et al. 1958; Silver et al. 1963). It may therefore become progressively more difficult for a whitefish egg resting on the sediment of a eutrophic lake to satisfy its increasing oxygen requirements. During the early stages of development, an egg can survive temporarily low ambient oxygen concentrations by reducing its oxygen consumption and slowing down development. At more advanced stages, however, a continual oxygen deficit can result in the death of the embryo.

The thickness of the DBL over the sediment decreases with increasing current speed. Laboratory measurements conducted by Jørgensen and Revsbech (1985) on coastal marine sediment yielded DBL thicknesses of 1.1 mm at low flow velocity (1–2 $cm \cdot s^{-1}$) and 0.5 mm at high flow velocity (10–20 $cm \cdot s^{-1}$). The DBL thickness of 1.5–2.5 mm estimated on the basis of oxygen measurements over sediment cores, where the current speed is actually zero during the measurements, might therefore be thought to overestimate the in situ value. However, computations show that this measured thickness is indeed likely to reflect in situ conditions. The thickness δ_g of the DBL for a gas is related to the thickness δ of the viscous sublayer as follows (e.g., Daykin 1965):

$$(6)\quad \delta_g = \delta Sc^{-1/3}$$

where Sc, the Schmidt number of the gas, is defined as the ratio of the kinematic viscosity ν to the molecular diffusivity of the gas at the ambient temperature. Setting $u^*\delta/\nu = 11.1$ (Chriss and Caldwell 1984) yields δ_g as a function of τ:

$$(7)\quad \delta_g = 11.1\ \nu\rho^{1/2}\ Sc^{-1/3}\ \tau^{-1/2}.$$

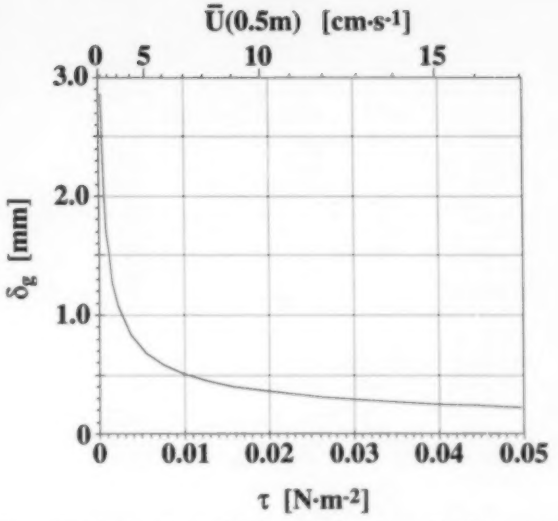

FIG. 8. Thickness of the diffusive boundary layer (δ_g) for oxygen over the sediment, at 4.5°C, as a function of shear stress (τ) (eq. (7)) and of current speed 0.5 m above the sediment surface ($\bar{U}$(0.5 m)) (eq. (3) and (7)).

Using the empirical formula of Himmelbau (1964) for the temperature dependence of molecular diffusivity, Sc for oxygen varies from 1240 at 4°C to 1165 at 5°C. Thus, at the lake temperatures prevailing in winter, the thickness of the oxygen DBL is 9.4% of that of the viscous sublayer, and the coefficient 11.1 $\nu\rho^{1/2}\ Sc^{-1/3}$ in eq. (7) takes on the value 5.1×10^{-5} $kg^{1/2} \cdot m^{1/2} \cdot s^{-1}$. The relationship represented by eq. (7) is illustrated in Fig. 8. (If required, δ_g can in general be computed from the mean current velocity $\bar{U}(z)$ measured at any given depth z by combining eq. (7) with eq. (3).) The mean current speed before the occurrence of the storm (i.e., 11–23 January 1990) was 2.0 $cm \cdot s^{-1}$, corresponding to a shear stress of 0.001 $N \cdot m^{-2}$. This gives a value δ_g = 1.6 mm, comparable with the DBL thickness determined experimentally (Fig. 7). Thus, under "normal" wind conditions, when the eggs are static, more than half of the surface area of each whitefish egg can be assumed to be within the oxygen DBL of the sediment. During calm conditions, the eggs can be assumed to lie entirely within the oxygen DBL.

The topography of the sediment surface is irregular, consisting of a landscape of "mountains" and "valleys". Three-dimensional measurements conduced in situ at the sediment–water interface at a depth of 15 m in a coastal marine environment by Gundersen and Jørgenson (1990) showed the DBL to be thinner over the "mountains" and thicker over the "valleys". The location of an egg on the sediment surface could therefore be of great importance for its chances of survival. In particular, a tendency to collect in depressions in the sediment surface after transport by currents is likely to be disadvantageous owing to the greater thickness of the DBL there. This disadvantage will be exacerbated by the fact that coarse organic material exhibits a similar tendency.

Conclusions

Naturally spawned whitefish eggs do not survive to hatch in eutrophic Lake Sempach. Storms occurring each winter

result in bottom currents over the spawning grounds in the littoral zone (Fig. 4) that are strong enough to transport eggs into deeper parts of the lake, where environmental conditions are less favourable for survival. The critical shear stress τ_c predicted to initiate egg transport ($0.02\ N{\cdot}m^{-2} \leq \tau_c \leq 0.04\ N{\cdot}m^{-2}$) will cause simultaneous resuspension of inorganic sediment of grain size less than about 10 μm (i.e., fine silt and clay), and subsequent differential settling will result in partial burial of the eggs ("egg sandwich"). Burial in the sediment increases the egg mortality rate firstly by forming a physical barrier that hinders the transport of oxygen to the egg and secondly by relocating the eggs in a zone of steep oxygen gradients and low oxygen concentrations (the upper few millimetres of sediment).

Those translocated eggs not buried in the sediment will lie within the oxygen diffusive boundary layer and will be subjected to oxygen concentrations which, although not zero, are still below the minimum required for survival to hatching (Fig. 7). The thickness of the oxygen diffusive boundary layer is comparable in size with, or greater than, the diameter of a whitefish egg over the range of current speeds likely to be encountered during "normal" conditions when wind and current speeds are low to moderate (Fig. 8). In POC-rich eutrophic lake sediments, a steep oxygen concentration gradient across the sediment–water interface exists, giving rise to low oxygen concentrations in the DBL. However, high ambient oxygen concentrations are required by the eggs during the more advanced stages of development. Thus, in eutrophic lakes with high surface sediment POC concentrations, the translocation of coregonid eggs to deeper regions, whether or not coupled with burial, is likely to result in egg mortality.

Acknowledgments

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Probability of First Sexual Maturation of Male Parr in Wild Sea-run Brown Trout (*Salmo trutta*) Depends on Condition Factor 1 yr in Advance

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Bohlin, T., C. Dellefors, and U. Faremo. 1994. Probability of first sexual maturation of male parr in wild sea-run brown trout (*Salmo trutta*) depends on condition factor 1 yr in advance. Can. J. Fish. Aquat. Sci. 51: 1920–1926.

In three years, in late autumn, underyearling wild sea-run brown trout (*Salmo trutta*) in a small stream were captured with electric fishing and tagged with microchips (passive integrated transponders). They were recaptured during the following season either as migrating smolts in a trap or by electric fishing in the stream just after the migration period and during spawning (late autumn). At spawning, they were distinguished as sexually mature male parr or immature juveniles. The smolts were longer and heavier at tagging than the mature male parr and the immature parr, which were similar in initial length and weight. The mature male parr had a significantly higher condition factor at tagging than the immature parr. The probability of parr maturation was positively associated with initial condition factor but not with initial body length. Growth of mature male parr and immature parr was similar in the early season but higher for immature parr in the late season. The result indicated that the choice of strategy was made earlier in life than previously recognized.

Pendant trois ans, à la fin de l'automne, des truites brunes (*Salmo trutta*) anadromes sauvages de moins d'un an ont été capturées par pêche électrique dans un petit cours d'eau et ont été équipées d'un micro-émetteur (transducteur passif intégré). Elles ont été capturées à nouveau la saison suivante à l'état de smolts en migration au moyen d'un piège ou au moyen d'un appareil de pêche électrique dans le cours d'eau juste après la migration et durant la période de fraye (à la fin de l'automne). Au moment de la fraye, elles ont été classées comme tacons mâles sexuellement matures ou comme juvéniles immatures. Les smolts étaient plus longs et plus lourds au moment du marquage que les tacons mâles matures et les tacons immatures dont la longueur et le poids initiaux étaient similaires. Le coefficient de condition était beaucoup plus élevé chez les tacons mâles matures au moment du marquage que chez les tacons immatures. La probabilité de maturation des tacons a été corrélée positivement au coefficient de condition initial mais non à la longueur corporelle initiale. La croissance des tacons mâles matures et des tacons immatures était similaire au début de la saison, mais beaucoup plus élevée chez les tacons immatures à la fin de la saison. Les résultats indiquent que les facteurs présidant au développement se sont manifestés plus tôt pendant le cycle vital qu'on ne l'avait d'abord pensé.

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The variation in life-history traits within populations of Salmonidae is often large, especially among males in migratory stocks. Males may mature either at a large body size after migration and a period of growth at sea or at a small size earlier in life at the parr stage before (or without) migration while still in the nursery stream. Mature male parr are sometimes called "precocious males", or parr males. Male parr maturation in migratory salmonid species and populations is widespread and has been reported in *Oncorhynchus* species (Groot and Margolis 1991), but more usually in wild and hatchery populations of Atlantic salmon (*Salmo salar*) and sea-run brown trout (*S. trutta*). In these species the incidence of parr maturation in wild populations is highly variable (salmon: Myers et al. 1986; trout: L'Abée-Lund et al. 1990). The mature male parr exhibit similar secondary characters as the sea-run males (Rydevik 1988) and participate in the spawning of the sea-run individuals (Bohlin 1975; Campbell 1977; Jonsson 1985; Myers and Hutchings 1987). Whereas the sea-run males attempt to monopolize females by aggressive behaviour, the mature male parr use a cryptic, sneaking strategy, often hiding behind obstacles in the immediate vicinity of the female (Jones and King 1950).

There seem to be two main reasons for the interest in this phenomenon. One is that parr maturation is an unwanted complication in aquaculture. Parr maturation affects smolting negatively and may even inhibit it (e.g., Thorpe 1986; Dellefors and Faremo 1988; Lundquist et al. 1988; Foote et al. 1991) and is therefore of economic importance. The second reason is the question of the adaptive mechanisms for the evolution and maintenance of such dimorphic populations and the mechanism of strategy choice. One suggested mechanism for the male dimorphism is frequency-dependent average fitness for the two alternatives as a result of behavioural interaction between the large, aggressive and small, sneaking males. This would lead to a population-specific equilibrium proportion p^* of males adopting the strategy of parr maturation and the proportion $(1 - p^*)$ choosing anadromy without prior maturation, where p^* is determined

by the survival and reproductive success of the two alternatives (Bohlin et al. 1986; Myers 1986; Gross 1991). The individual choice of strategy can be based on conditions related to phenotypic characters (growth rate, size at certain age, nutritional state, etc.). This kind of mechanism, a mixed conditional evolutionary stable strategy (ESS) (Maynard Smith 1982), has been suggested for the choice of reproductive strategy for males in migratory populations of salmonids (Leonardsson and Lundberg 1986; Bohlin et al. 1990). The conditions determining the choice are, however, far from clear.

Several studies of Atlantic salmon indicate that body size and nutritional state are associated with parr maturation and may be used as cues for the choice of strategy. Evidence that body length in the first winter is positively associated with parr maturation in the following autumn has come from tagging fish in the laboratory or back-calculating scales in wild fish (Baglinière and Maisse 1985; Myers et al. 1986; Rowe and Thorpe 1990a; Berglund 1991; Prévost et al. 1992; Simpson 1992). Condition index (weight scaled to length) prior to male parr maturation has only been studied under laboratory conditions, indicating that condition index is similar for future mature male parr and nonmaturing individuals in the first winter; later in the following season the mature male parr have higher condition (Rowe and Thorpe 1990a; Berglund 1991; Herbinger and Friars 1992). Mature male parr also have generally higher lipid content throughout the season (Rowe et al. 1991; Simpson 1992). In laboratory experiments with restricted feeding during spring when the gonads start to develop, the incidence of parr maturation was reduced (Rowe and Thorpe 1990b; Berglund 1991); similar experiments in late winter did not have this effect (Herbinger and Friars 1992). Growth rates for mature male parr and immature parr under laboratory conditions appeared to be similar in the early season (Rowe and Thorpe 1990a; Berglund 1991; Simpson 1992); towards spawning time in the late season the immature parr grow faster.

Although the above results from hatchery populations may be relevant also for wild fish, conditions in the hatchery are different from those in nature with respect to many factors that could affect life history. Growth rates and condition factors in the laboratory are usually higher, population density is higher with effects on social interactions, predators are absent with possible effects on foraging behaviour, etc. Studies on wild populations have long been prevented by the lack of tagging methods suitable for small fish; such methods are now available.

In this paper, we report the result of a study of wild, one-summer-old juveniles of sea-run brown trout, tagged in late autumn with microchips and recaptured during the following season. The main objective was to investigate whether the probability of parr maturation depends on body size and condition in the preceding year.

Methods

The tagging was conducted during 1990–92 in Norumsån, a small stream (mean width 2.4 m, length 8.5 km) in southwestern Sweden (latitude 58°N). During October–December, one-summer-old sea-run trout were captured by electric fishing on a 500-m stream site, anesthesized with 2-phenoxyethanol, measured to the nearest millimetre, weighed to the nearest 0.1 g, and checked for male sexual maturation (release of milt). A total of 1048 individuals from the upper tail of the length–frequency distribution were tagged (size ranges: 1990, 65–85 mm; 1991, 65–100 mm; 1992, 65–90 mm); fish smaller than 65 mm were judged too small to tag. The tags were passive integrated transponders (PIT tags) (type TX1400, Destron/IDI). A small cut through the skin was made using a sharp scalpel through which the tag was inserted into the posterior part of the body cavity using a veterinarian syringe. To distinguish tagged from nontagged fish, the adipose fin was removed on all tagged individuals.

The fish were recaptured during the following season either as migrants in a smolt trap situated 1 km below the site (about 5 mo after tagging, mainly in April–May) or by electric fishing in the stream after the smolt season in two periods: in June (after about 6 mo) or at the end of the season (approximately 1 yr after tagging). The electric fishing for recapture was conducted on the same section as when tagging plus 50 m upstream and 150 m downstream. In the last period, the recaptures were distinguished either as immature parr or as sexually mature male parr. Length, weight, and sexual state at recapture were recorded as at tagging.

All statistical tests were performed using SAS (1988), either the GLM process for analysis of variance (ANOVA) and analysis of covariance (ANCOVA) or the CATMOD process for logistic regression. For pairwise comparison of means in the ANOVAs, the Tukey–Kramer option was applied. The following variables were analyzed: L_0, length (millimetres) at tagging; W_0, weight (grams) at tagging; C_0, condition (weight relative length) at tagging; L_1, length (millimetres) in June; W_1, weight (grams) in June; C_1, condition (weight relative length) in June; GL_{01}, growth in length from tagging to June ($GL_{01} = \log(L_1/L_0)$); GW_{01}, growth in weight from tagging to June ($GW_{01} = \log(W_1/W_0)$); L_2, length (millimetres) at the end of the season; W_2, weight (grams) at the end of the season; C_2, condition (weight relative length) at the end of the season; GL_{12}, growth in length from June to the end of the season ($GL_{12} = \log(L_2/L_1)$); GW_{12}, growth in weight from June to the end of the season ($GW_{12} = \log(W_2/W_1)$). As only the upper tail of the length–frequency distribution was tagged, length L_0 and weight W_0 were not normally distributed. Since there is no standard transformation for this type of distribution, we tried $\log(x - k)$, where k was adjusted until the distributions were approximately normal. The best results were obtained for the transformations $\log(L_0 - 60)$ and $\log(W_0 - 2)$, respectively (Fig. 1), which were used in the ANOVAs.

The condition C_i was the residuals from the linear length–weight relation $\log(W_i)$ on $\log(L_i)$. We used ANCOVA in a model of the form

$$\log(W_i) = \log(L_i), \text{ year}, \log(L_i) \times \text{year}$$

where $\log(L_i)$ is the covariate, year a class variable, and $\log(L_i) \times$ year the interaction used to test for slope homogeneity among years.

By two-way ANOVAs, we tested the homogeneity of means for the variables x above. The treatments were year, with levels 1991, 1992 and 1993, and strategy (strat). The levels of strat were I (=those still immature parr at the end of the period), M (=sexually mature male parr at the end of the period), and S (=migrants during the period) for state at tagging and levels I and M for state in June and at the end of the season. The general form of the ANOVA was

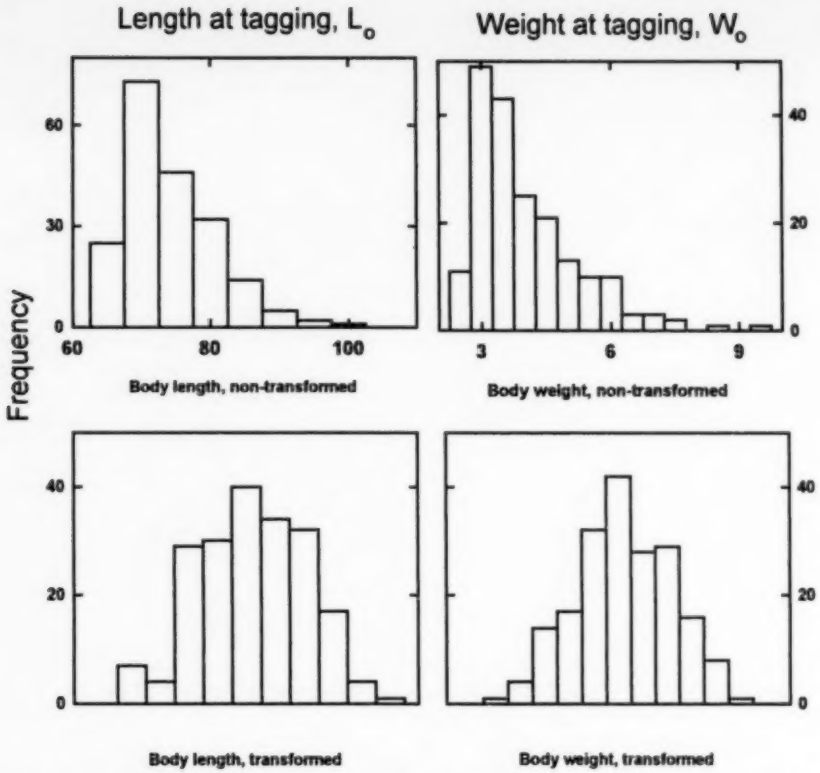

FIG. 1. Frequency distribution of body length (L_0) and body weight (W_0) at tagging for original and transformed variables.

TABLE 1. State at tagging for the variables body length L_0 (mm), body weight W_0 (g), and condition factor C_0 for the strategies immature parr (I), mature male parr (M), and smolts (S), together with the result of two-way ANOVA with classes strategy (I, M, or S) and year. p_{strat} is the probability for homogeneity of means among strategies and R^2_{strat} the proportion of the total variance explained by strategy. $p < 0.05$ among pairs is the result of the Tukey–Kramer pairwise comparison.

	Means within strategy					
Variable	I (n = 111)	M (n = 61)	S (n = 26)	p_{strat}	R^2_{strat}	$p < 0.05$ among pairs
L_0 (mm)	71.99	73.25	75.77	0.0181	0.042	S–I
W_0 (g)	3.55	3.95	4.25	0.0021	0.064	S–I, M–I
c_0·100	−1.932	3.383	0.657	0.0005	0.079	M–I

x = strat, year, strat × year

where strat and year are class variables (main effects) and strat × year the interaction.

Results

None of the trout was sexually mature at tagging. Of the 1048 one-summer-old trout tagged, 198 (18.9%) were captured during the following season. Of these, 26 were caught as smolts in spring (strategy S), 61 in late autumn as mature male parr (strategy M), and 111 in late autumn as immature parr (strategy I). Of strategy M, 28 were also recorded in the June sampling, and of strategy I, 57 were also recorded in the June sampling.

For state at tagging (variables L_0, W_0, C_0) the ANOVA gave no significant effect of year or the interaction year × strat, so we used only the main effects. The effect of strategy was significant (Table 1). The pairwise comparison indicated that the subsequent smolts were longer and heavier than the remaining immature parr in the stream and also heavier than the mature male parr (Table 1). The mature male parr were not significantly longer or heavier than the immature parr, but they had higher condition and were thus on average heavier in relation to their length (Table 1;

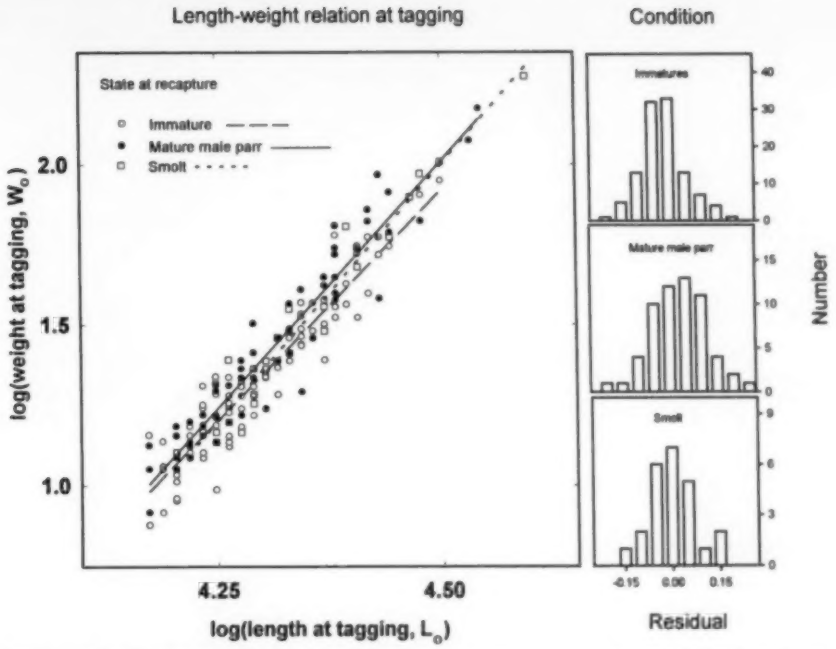

FIG. 2. Left panel: relation between weight W_0 and length L_0 at tagging in double-logarithmic scale for immature parr, mature male parr, and smolts. In the graph, the effect of years is eliminated using ANCOVA. Right panels: condition factor at tagging, expressed as (residuals from the length–weight relations) × 100, for individuals that, in the following season, remained immature, became mature male parr, and migrated as smolts.

Fig. 2). The difference in condition between strategies I and M corresponded to about 5% in weight for the same length. Generally, however, strategy explained only a small fraction of the total variance (Table 1).

Our data thus indicated a positive relation between the probability of becoming sexually mature at the parr stage and the initial condition factor, and possibly also initial body size. The hypothesis that the categorical response variable "choice of strategy" (M or I) was independent of condition, body size, and year, including interactions, was explicitly tested by logistic regression using the model

Probability for M = condition, body length, year

and all possible interactions. None of the interactions was significant, so we used only the main effects. Condition had a significant positive effect on the probability of choosing strategy M ($p = 0.0003$); for body length the effect was positive but not significant ($p = 0.2231$), and year had no effect ($p = 0.4661$).

In the June sampling, 28 future mature male parr (from the M group) and 57 immature parr (from the I group) were recaptured. The ANOVAs did not show significant effects of strategy for any of the June values of the variables length, weight, or condition or for growth in length or weight from June to the end of the season (Table 2). Thus, in contrast with the situation at tagging, we found no difference in condition factor C_1; in June the future mature male parr were not significantly heavier for the same length than those remaining immature parr. This may have been due to a smaller sample size in June than at tagging. However, when we used this restricted sample of individuals to test their initial condition factor C_0, we found a significant effect of strategy ($p = 0.0380$). This suggested that the parr following the two strategies really were more similar with respect to condition in June than in the previous late autumn.

TABLE 2. State in June (subscript 1) and in the following autumn (subscript 2) for the variables body length L (mm), body weight W (g), and condition factor C and growth autumn–June (subscript 01) and June–autumn (subscript 12) for length (GL) and weight (GW) together with the result of two-way ANOVA with classes strategy (I or M) and year. p_{strat} is the probability for homogeneity of means among strategies and R^2_{strat} the proportion of the total variance explained by strategy (given only in the case of significant difference of means).

	Means within strategy			
Variable	I	M	p_{strat}	R^2_{strat}
L_1 (mm)[a]	104.30	106.08	0.2897	—
W_1 (g)[a]	12.69	13.62	0.1921	—
$c_1 \cdot 100$[a]	−0.044	0.089	0.9302	—
GL_{01}[b]	0.3560	0.3541	0.8975	—
GW_{01}[b]	1.2214	1.1792	0.4066	—
L_2 (mm)[a]	130.90	129.54	0.3573	—
W_2 (g)[a]	20.80	22.27	0.0868	—
$c_2 \cdot 100$[a]	−3.022	6.187	0.0001	0.340
GL_{12}[b]	0.2249	0.1910	0.0008	0.126
GW_{12}[b]	0.4940	0.4815	0.7964	—

[a] $n = 111$ for I and $n = 61$ for M.
[b] $n = 57$ for I and $n = 28$ for M.

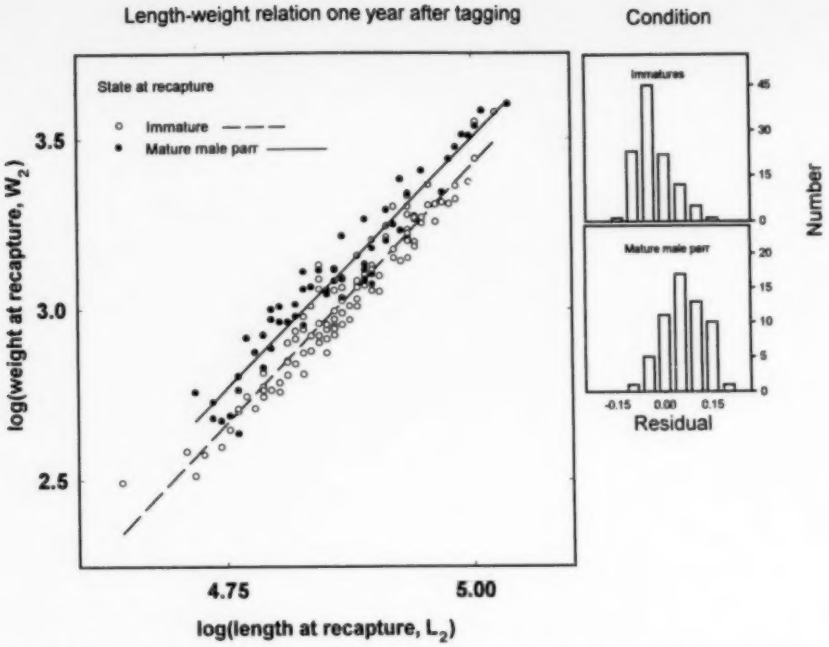

FIG. 3. Left panel: relation between weight W_2 and length L_2 1 yr after tagging (in spawning time) in double-logarithmic scale for immature parr and mature male parr. In the graph, the effect of years is eliminated using ANCOVA. Right panels: condition factor, expressed as (residuals from the length–weight relation) × 100, for the two categories.

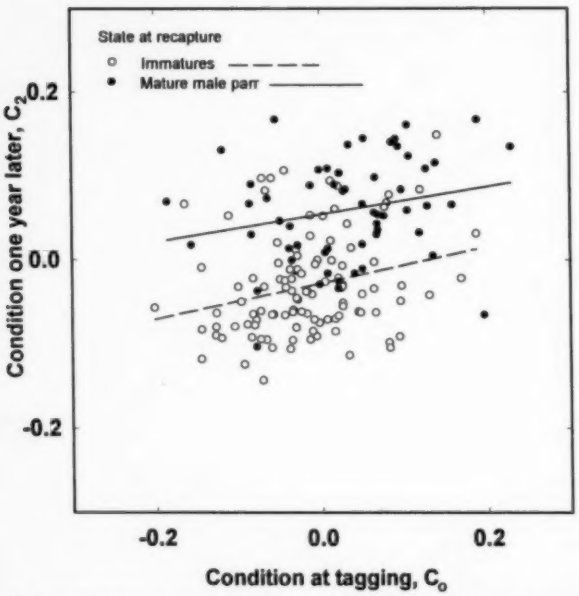

FIG. 4. Relation between condition factor C_0 at tagging and condition factor C_2 1 yr later (at spawning time) for immature parr and mature male parr. The relation is positive for both categories, and mature male parr have, on the average, a higher condition on both occasions. In the graph, the effect of year is eliminated using ANCOVA.

At the end of the season, we found no differences in length, weight, or growth in weight from June between the mature male parr and the immature parr (Table 2). The growth in length was, however, higher for the immature parr than for the mature male parr (Table 2), and there was also a quite substantial difference in condition C_2; the mature male parr were about 10% heavier for the same length than the immature parr (Table 2; Fig. 3). Strategy explained 34% of the total variation in condition. Another question was whether the condition factor C_2 at the end of the period for an individual depended on its condition C_0 at tagging (1 yr previously). We tested this using ANCOVA with strategy and year classes and including interactions. The interactions were not significant, so we included only the main effects. We found a positive relation between C_2 and C_0 ($p = 0.0004$) with slope 0.33 (Fig. 4). It thus appears that an individual heavier than average for its length in one year was still, on the average, heavier than average for its length 1 yr later.

Discussion

By following wild, individually tagged, young-of-the-year trout juveniles over the following season, we concluded that those recaptured as migrating smolts were initially bigger than those not migrating and that those ending up as sexually mature male parr were initially heavier in relation to their length than those still juvenile at the end of the season. It was also evident that the immature parr grew faster in length after June than the maturing male parr; before June, we found no difference between these groups. However,

when evaluating our results, the three following points should be considered.

The first is that we could not exclude the possibility that the difference in initial body size between smolts and parr was affected by size-dependent efficiency of the smolt trap; the grid in the trap was 10 mm wide, so fish smaller than about 90 mm in length may not have been efficiently captured. The result, however, was well in agreement with previous studies on smolting in Atlantic salmon. In many hatchery-reared populations of this species, the cohort tends to develop a bimodal length distribution towards the end of the first season, in which fish in the upper mode are those that exhibit smolt characteristics the following spring (e.g., Thorpe 1977). The occurrence of bimodality in wild populations is less well established, but may be a real phenomenon also in nature (Heggenes and Metcalfe 1991). In our population, we did not observe bimodality, either because it did not exist or because the proportion migrating after 1 yr was low and masked by the variance within size groups.

The second point is that we could not determine the sex of the immature parr, which thus included both females and immature males. The most relevant comparison would have been that between males of the two groups M and I. Immature males and females in Atlantic salmon are, however, similar with respect to size, condition, and growth (Rowe and Thorpe 1990a; Berglund 1991; Simpson 1992). If the situation is similar for sea-run trout, the inclusion of females in the I group is not likely to affect our conclusions but will contribute to increased unexplained variance in the comparisons.

The third point of concern is that only the largest young-of-the-year individuals were tagged, so our conclusions are valid only for this segment of the cohort. If the entire cohort had been possible to include, our result regarding the relation between initial body size and choice of strategy might have been different. Other studies have indicated that body size in the first winter is positively associated with parr maturation in Atlantic salmon (Baglinière and Maisse 1985; Myers et al. 1986; Prévost et al. 1992). It is possible that the situation is similar for sea-run brown trout; if there is a low first-winter body length threshold for parr maturation (below 65 mm), we would not have detected it.

In some respects, our results concerning the difference between immature parr and mature male parr were similar to those obtained for hatchery-reared Atlantic salmon: growth was similar in spring but lower for the mature male parr in summer–autumn, and the condition index was higher for mature male parr in the late season (Rowe and Thorpe 1990a; Berglund 1991; Herbinger and Friars 1992; Simpson 1992).

The most notable result was the association between the condition factor in late autumn and the probability of parr maturing 1 yr later. Such a tendency has not been demonstrated previously; the condition factor in the first winter appears to be similar for mature male parr and immature parr in hatchery populations of Atlantic salmon, or possibly is even higher for immature parr (Rowe and Thorpe 1990a; Berglund 1991; Herbinger and Friars 1992; Simpson 1992). This discrepancy may be due to differences in condition (hatchery nature), species (salmon or trout), or other less obvious factors. One interpretation of the result is that the choice of strategy in wild trout was affected by environmental factors at the end of the first season or earlier. This does not exclude the possibility that later conditions, e.g., in late spring or early summer (Rowe and Thorpe 1990b; Berglund 1991), also can modify the choice, but that the possible effect of these was not sufficiently strong to disguise the relation we found. Another interpretation is that parr maturation is at least partly heritable, and thus that the high condition factor for mature male parr in their first autumn was a part of the mature male parr strategy (like a secondary sex character) rather than related to its cause. Parr maturation in Atlantic salmon has been shown to be partly under genetic control (Naevdal 1983; Thorpe et al. 1983; Gjerde 1984), although there is a strong environmental component (Rowe and Thorpe 1990b; Berglund 1991). This indicates the existence of genetic intrapopulation variation in the conditional program coding for choice of strategy; such variation may include the age or size at which the choice is made, the cue variable(s) utilized, and the threshold value(s) of the cue(s) required for strategy modification. This study supported the view that the choice of parr maturation in wild sea-run brown trout was made earlier in life than has been suggested for hatchery-reared salmon (Rowe and Thorpe 1990b; Berglund 1991), either because of heritability for parr maturation or as a result of nutritional state early in life. Body size may also be involved, even if we found no relation; the results from Baglinière and Maisse (1985), Myers et al. (1985), and Prévost et al. (1992) showed that the within-population variation (among year or stream sections) in probability of parr maturation was partly explained by body size.

However, although there obviously is some consensus that nutritional state and/or body size (or variables related to them) is of importance for the choice of strategy, there seems still to be some confusion as to how these states are expected to affect the probability of choosing the strategy of parr maturation in natural populations. If this probability p is maintained as a mixed conditional ESS, one would expect a dependence within populations (e.g., among years or stream sections) of p and the phenotypic distribution, as, for example, a positive dependence between average body size for immature yearlings and mature male parr frequency in the same cohort in the following autumn. One would, however, not expect a similar dependence among populations even if the characters utilized as cues for the choice were the same; the long-term equilibrium probability of parr maturation is expected to be set by other ecological parameters, primarily the reproductive success and survival of anadromous and resident parr males (Bohlin et al. 1990). This view was supported by the results from Myers et al. (1986) in Atlantic salmon; they found that body size partly explained the intrapopulation variation, but not the interpopulation variation, in male parr frequency. Analyses of factors affecting the interpopulation variation in the probability p of choosing parr maturation are, however, made difficult by the fact that the terminology is ambiguous. Several authors have used the ratio R = (no. of mature male parr)/(no. of immature male parr + no. of mature male parr) among all age classes in the stream population in this context. It is to be noted that R is not an estimate of p. The ratio R depends, by definition, primarily on age at maturation; if age at parr maturation is high, the proportion of mature parr males would be low, even if all males eventually matured at the parr stage ($p = 1$). This shows that the ratio R tells little of the probability p of parr maturation as opposed to the probability $(1 - p)$ of migration without prior maturation. The ratio R of mature

male parr frequency among populations was positively associated with growth rate in sea-run brown trout (L'Abée-Lund et al. 1990), at least partly as a result of higher age at maturation in more slowly growing populations. There is, however, no evidence of such a dependence between the probability p and growth; slower growth appears to be associated with later choice between parr maturation and anadromy, but not necessarily for decreased probability of parr maturation (Bohlin et al. 1990).

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Fidelity of Anadromous Arctic Char (*Salvelinus alpinus*) to Nauyuk Lake, N.W.T., Canada

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The estimated fidelity of anadromous Arctic char (*Salvelinus alpinus*) returning to Nauyuk Lake from 1974 to 1978 was low (53%). Seven percent of the fish were absent for 1 or 2 yr before returning. Fidelity was also size dependant. Intermediate-sized fish (35–65 cm) had the highest estimated fidelity, while smaller and larger fish had lower rates. The char leaving Nauyuk Lake each spring were categorized into three groups: postspawning char, char that had been observed returning to Nauyuk Lake at least once, and char that had not shown any fidelity to Nauyuk Lake in the past. The char that had shown fidelity to Nauyuk Lake previously had the highest fidelity rate (75%), while the postspawning char and those that had not shown any previous fidelity to Nauyuk Lake both had a fidelity rate of 45%. The low fidelity of the postspawning char was attributed to a higher mortality rate. Fidelity could not be correlated with the length of time a fish was at sea or when it left Nauyuk Lake in the spring.

La fidélité de l'omble chevalier (*Salvelinus alpinus*) anadrome retournant au lac Nauyuk de 1974 à 1978 a été estimée à 53 %, ce qui est faible. Sept pour cent des individus sont restés absents 1 ou 2 ans. On a également constaté que la fidélité est liée à la taille de l'individu. On estime que le taux de fidélité était le plus élevé chez les poissons de taille moyenne (35–65 cm), alors qu'il était plus bas chez les les poissons de grande et de petite tailles. Les ombles chevaliers qui quittent le lac Nauyuk chaque printemps ont été classés en trois groupes : les ombles post-fraye, ceux qui sont remontés au lac au moins une fois et ceux qui n'ont montré aucune fidélité au lac dans le passé. Les ombles qui avaient été fidèles au lac auparavant ont montré le plus haut taux de fidélité (75 %), alors que les ombles post-fraye et ceux qui n'avaient montré aucune fidélité antérieurement avaient un taux de fidélité de 45 %. Le faible taux de fidélité de l'omble post-fraye est attribué à un taux de mortalité plus élevé. On n'a pu établir aucune corrélation entre la fidélité d'un sujet et le temps qu'il passe en mer ou le moment où il quitte le lac au printemps.

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The management of Arctic char (*Salvelinus alpinus*) in the Canadian Arctic is based on the assumption that each river system has a discrete stock (Kristofferson et al. 1984). Fisheries typically take place during anadromous migrations as the char either move into the sea to feed in the spring or return to freshwater in the fall to overwinter. Quotas are assigned on a river-by-river basis. Inherent in this "discrete stock" management strategy is the assumption that char have a high fidelity rate to their natal stream. The fidelity rate is defined as the probability that a fish leaving a freshwater system in the spring will return to that same system in the fall. Some evidence supports this assumption (Grainger 1953; Nordeng 1961; Glova and McCart 1974; Moore 1975). However, more recent studies have shown that this assumption may not be valid in all cases (Jensen and Berg 1977; Johnson 1980; Gyselman 1984). If there is a significant exchange of fish among river systems, then quotas assigned to individual rivers have little biological meaning and the concept of a "stock", the catchable portion of a discrete population (Ricker 1975), must be reexamined.

In 1973, the Fisheries Research Board of Canada, now the Department of Fisheries and Oceans, began a long-term study on the population dynamics, energetics, and migrations of Arctic char at Nauyuk Lake (68°22′N, 107°35′W) in the central Canadian Arctic. Johnson (1980, 1989) and Gyselman (1984) have published overviews of this research. As part of this study, a tagging program was carried out that enabled a detailed examination of fidelity in the Nauyuk Lake system. Initial results showed that the fidelity rate was not as high as expected (Gyselman 1984) and a more detailed analysis was warranted.

In this paper, the results of the tagging study as they apply to fidelity are presented. The overall fidelity rate for the sea-run "stock" is estimated and then a more detailed examination is made of the fidelity among three readily identifiable groups within the "stock": postspawning char, char that had previously shown fidelity to the Nauyuk Lake system, and char that had not shown any fidelity to the system previously. The objective was to determine whether the Nauyuk Lake "stock" is homogenous or a composite of separate groups of char with different fidelity rates. Fidelity rates are also compared among size classes and between years to determine the range of fidelity that might be expected in other river systems within the central Canadian Arctic.

Materials and Methods

Background and Life History

The Nauyuk Lake system is ideal for the study of Arctic char because of the clear separation of life-history stages. The

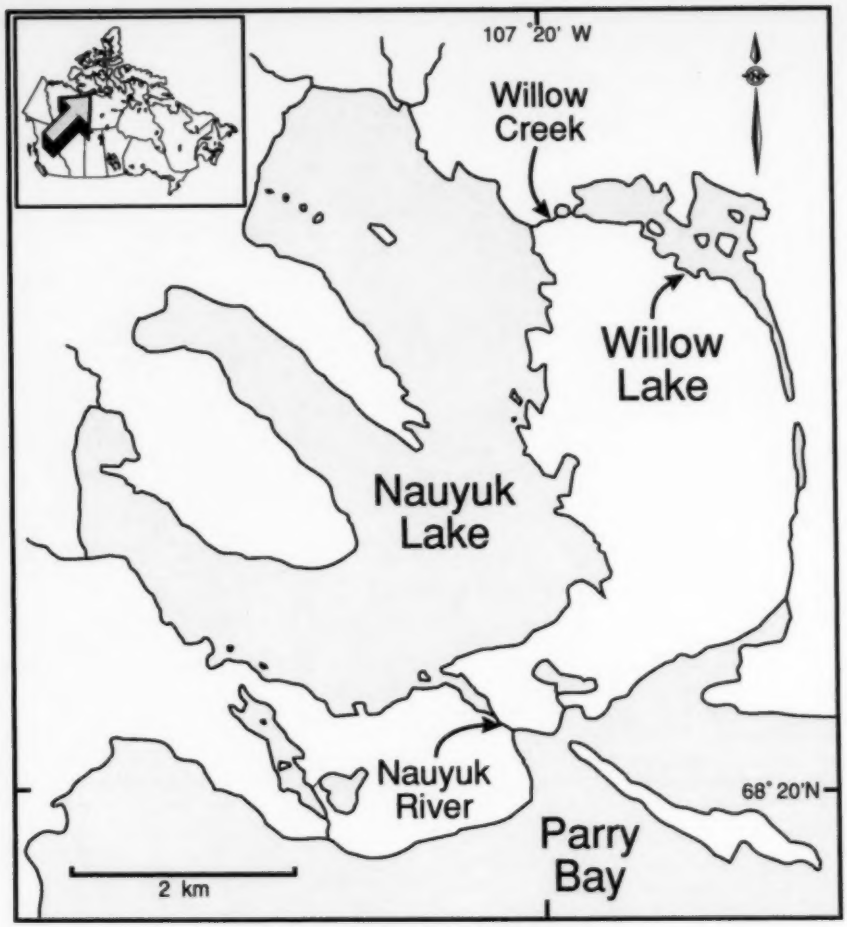

FIG. 1. Nauyuk Lake study area.

life cycle of char in Nauyuk Lake is described in detail in Johnson (1989); only a brief summary is necessary here to define the details important to this study.

Nauyuk Lake is connected to the sea (Parry Bay) by the Nauyuk River (Fig. 1). The Nauyuk River is short (200 m) and char can pass upstream and downstream easily even in low-water years. Each spring, as soon as the river starts to flow, the downstream run begins. Larger char tend to migrate early. The mean daily length decreases throughout the run until finally, at the end, smolts migrate to sea for the first time. After spending 35–45 d at sea, the char start to migrate back upstream to overwinter in Nauyuk Lake. In most years, there is a clear separation of the downstream and upstream runs with at least a few days in the middle of the summer when no fish are moving either up or down the Nauyuk River.

Prespawning char that have overwintered in Nauyuk Lake do not go to sea in the spring of years they spawn. Instead, they migrate up Willow Creek (Fig. 1) into Willow Lake. Spawning in the Nauyuk Lake system appears to be confined to Willow Lake. To date, no spawning sites have ever been found in Nauyuk Lake itself and prespawning char are very rare in the upstream run in the Nauyuk River. Willow Creek is narrow and braided. Passage is only possible during the spring melt. Maturing char spend the summer in Willow Lake, spawn in the fall, overwinter, and then migrate back down Willow Creek the following spring. A small number (<5%) spend 2 yr in Willow Lake, but whether these fish spawn in both years has not been determined. Therefore, two migrations take place simultaneously in Willow Creek each spring. The prespawners migrate upstream, while the postspawners from the previous year migrate downstream. After leaving Willow Lake, postspawners immediately migrate to sea via the Nauyuk River. A detailed chronology of the migrations is presented in Gyselman and Broughton (1991). The segregation of the spawning and nonspawning char into two discrete runs enables a detailed study of each of these life stages.

Sampling Procedure

A weir was constructed across the head of the Nauyuk River each spring from 1974 to 1979 to monitor the downstream migration. When the downstream run was complete, the weir was reconfigured to study the upstream run. In 1979, only the downstream run was monitored.

Ice from Nauyuk Lake was a constant problem early in the spring, often damaging the weir and allowing some escapement seaward. Escapement was usually small (<5%), but in 1976 the weir could not be installed until well after the

run had begun and less than 15% of the char were counted. Similarly, in 1974 the weir was installed late and only about 35% of the run was sampled. However, escapement was not a problem during this study. It was estimated from the return of tagged fish that were recorded going upstream the previous fall and recorded again going upstream in the study year, but had not been seen migrating downstream in the spring of the study year.

The weir was covered with wire cloth with 1.5-cm mesh openings. This allowed the capture of all char, including smolts. All fish in the runs were counted and a subsample was weighed and measured (fork length). Every attempt was made to ensure that this subsample was random and representative of the population as a whole. The time of day the subsamples were taken was varied and the subsamples were as large as possible (100–300 fish·d^{-1}). All of the fish migrating during a prescribed time period were sampled so that no length bias was introduced. At the beginning and end of the run, when the number of fish migrating per day was low (<100 fish·d^{-1}), all of the fish were sampled.

As many char as possible were tagged (25–75 fish·d^{-1}) within the logistic constraints of other project requirements. Each tag carried a unique code so that when subsequently recaptured, measurements could be related to the known history of each fish. Floy anchor tags (Floy Tag and Manufacturing Inc., Seattle, Wash.) were used on fish over 35 cm and Carlin tags (Howitt Plastics Co., Molalla, Oreg.) attached with hollow nylon thread on fish less than 35 cm. The size distribution of tagged fish was not representative of the whole population because certain size groups were often selected for tagging to achieve other project objectives. Therefore, adjustments had to be made in an attempt to provide a better representation of the fidelity of the study groups. These adjustments are discussed below.

In Willow Creek, a two-way weir was built so that both the pre- and postspawning runs could be monitored at the same time. Again, wire cloth with 1.5-cm mesh openings was used to cover the weir. All mature char and smolts migrating both upstream and downstream in Willow Creek were tagged, weighed, and measured. The same tag types and length criteria for tag choice used at the Nauyuk River were used at Willow Creek.

Analyses

Length bias of tagged char

The first step in the analyses was to convert the known bias in the length distribution of tagged char migrating in the Nauyuk River to an unbiased estimate for the run as a whole. Tagged fish in the downstream run were assigned to groups based on 1-cm length intervals. The proportion of tagged fish returning was calculated for each length group by dividing the number of tagged fish in the group that returned in the fall by the number that left in the spring. The daily sample of all measured fish in the downstream run was assumed to be an unbiased estimate of the daily run as a whole. Therefore, fidelity of the particular length group was calculated by multiplying the number of char in the length group by the proportion of tagged fish in that length group that returned. However, the same proportion of fish in the daily run was not sampled each day. There was also a known decrease in mean length from the beginning to the end of the run. Therefore, daily totals in each length group were estimated by multiplying the total number of fish in the downstream run each day by the proportion of measured fish that were in each length group that day. The following equation describes the procedure:

$$(1) \quad f = \frac{\Sigma n_{lth}\, p_{lth}}{n_d}$$

where f = daily fidelity (pooled over all lengths), n_{lth} = number of char in length group lth migrating during the specific day, p_{lth} = proportion of tagged fish in length group lth that returned, and n_d = total number of char migrating downstream during the specific day d.

The numbers of char per day in each length group were summed by length group for the whole run to give overall totals for each length group. This total was multiplied by the proportion that returned for each length group to estimate the overall fidelity for that length group during the run. The overall fidelity for the whole run in each year was then calculated as the weighted average of the adjusted fidelities for all the length groups. This calculation is expressed by the equation

$$(2) \quad F = \frac{\Sigma N_{lth}\, p_{lth}}{N}$$

where F = overall fidelity for the run, N_{lth} = sum of the number of char in the particular length group each day for the whole run, p_{lth} = proportion of tagged fish in length group lth that returned, and N = total number of char in the run.

A separate fidelity conversion was done for each year. Data from Willow Creek did not need conversion because every fish was measured and tagged.

Tests of fidelity

The first analysis done tested the hypothesis that there was no significant difference in fidelity among the length groups, for all fish in the run. A randomized complete block design ANOVA compared the probability of returning among length groups pooled over years. Years were used as blocks to account for variation associated with temporal differences. An arcsine transformation was done to normalize the proportional data (Snedecor and Cochran 1967).

A second ANOVA tested for significant differences in fidelity between years. Length classes were used as blocks to account for variation associated with length differences.

To ensure that the results of the ANOVA for differences in fidelity among length groups were not related to the bias associated with the decline in size of fish through the downstream run, a regression of the probability of a fish returning versus when, during the run, it left Nauyuk Lake was calculated. Similarly, Johnson (1989) also noted that larger fish tended to stay at sea longer. Therefore, a second regression was calculated to compare the probability of returning versus length of time at sea.

Char that migrated down the Nauyuk River each spring could be readily identified as belonging to one of three groups: postspawning fish that were observed migrating down the Nauyuk River in the same spring as they were seen leaving Willow Lake (referred to as "postspawning" char), char that were not "postspawners" but had shown a degree of fidelity to the system by returning to Nauyuk Lake at least once in the past (referred to as "previously tagged" char), and char that had an unknown fidelity to the Nauyuk system because they were tagged for the first time

TABLE 1. Estimated net fidelity of Arctic char to Nauyuk Lake in the same year, after a 1-yr absence and after a 2-yr absence. Adjustments have been made for mortality and tag loss.

Year	Number tagged going downstream	Estimated proportion of downstream migrants returning		
		Same year	After 1 yr	After 2 yr
1974	789	0.344	0.064	0.051
1975	1075	0.498	0.075	0.013
1976	590	0.390	0.018	0.009
1977	2275	0.575	0.038	—
1978	1024	0.670	—	—
Total	5753			
Mean		0.526	0.048	0.024

leaving in the spring of the year in question (referred to here as "newly tagged" char). The two ANOVAs used on the pooled data testing for between-length differences and between-year differences were repeated for the "previously tagged" groups and "newly tagged" group. An analysis for the "postspawning" group was not necessary because all fish were tagged and measured. Finally, an ANOVA using length/years as blocks tested for an overall significant difference in fidelity between the "previously tagged" and "newly tagged" groups.

To visually display the differences in fidelity among the three groups, a polynomial regression was calculated and plotted for each group using the curve-fitting function in the SigmaPlot software package (Jandel Scientific, San Rafael, Calif.).

Biases in fidelity estimates

The estimates of fidelity discussed above are "gross" rates because they do not take into account either tag loss or mortality due to fishing, tagging, or natural causes. "Net" fidelity will be used when these losses are accounted for and is described by the following equation:

$$(3) \qquad F_{net} = F_{gross}(S_{nm}\, S_{fm}\, S_{tm}\, T)$$

where F_{net} = net fidelity, F_{gross} = gross fidelity, S_{nm} = survival rate from natural mortality, S_{fm} = survival rate from fishing mortality, S_{tm} = survival rate from tagging mortality, and T = tag retention rate.

Estimating losses leading to net fidelity

Net fidelity was calculated by subtracting losses due to natural, fishing, and tagging mortality and tag loss from gross mortality (equation (3)). While fishing mortality and tag loss could be measured in this study, there was no way of directly estimating natural and tagging mortality.

Most fishing mortality was limited to one local Inuit family fishing for domestic needs. However, char tagged at Nauyuk Lake were also caught at other fisheries (Gyselman 1984) although mortality from these sources was very small (<0.01%). All fish caught by the local Inuit were sampled and could be excluded from this study. Also, all fish that were known to have died from other causes such as bird predation were also eliminated. Therefore, gross fidelity figures already accounted for these losses.

Estimates of tag loss for anchor and Carlin tags in salmonids are very low (0–10%) (Armstrong 1965; Armstrong and Blackett 1966; Keller 1971; Butler and Loeffel 1972; Carline and Brynildson 1972). To verify these estimates, each char in the Nauyuk River runs was examined for a tag scar as it passed through the weir. Annual estimates of tag loss from scars were always less than 2%. However, tag scars could have been missed or other marks could have been interpreted as tag scars. Therefore, a double-tag experiment was started in 1976. One hundred fish were each tagged with two Floy tags. During the following years, each of these fish was observed to see if one of the tags had been lost. In no case was this observed. Therefore, tag loss was apparently very low, confirming the results of other studies. A conservative value of 2% was allowed for tag loss.

Natural mortality and tagging mortality cannot be directly measured when significant infidelity is suspected. If a tagged fish is never seen again, it is impossible to determine whether it died or emigrated. Therefore, natural and tagging mortality had to be estimated.

There are a number of estimates of natural mortality for Arctic char in the literature. Moore (1975) estimated annual natural mortality to be 16% for char in Cumberland Sound on Baffin Island. However, mortality seemed to be size selective, ranging from 5 to 30% depending on length. Nordeng (1961) estimated that the annual natural mortality rate in the Salangen River, Norway, was about 14%. Estimates for Creswell Bay range from 9.5 to 12% (Sekerak et al. 1976). Finally, Shershnev et al. (1986) estimated a somewhat higher rate of 24–26% for the Melkaya River in Russia, but this stock has a much shorter life cycle than those typically found throughout the Canadian Arctic.

Annual mortality can also be estimated from age–frequency data but only if fidelity is known to be high (Ricker 1975). Unfortunately, if fidelity is low, then the fish in each age cohort are not necessarily from the same stock and the validity of the estimate is in question. However, doing the calculation for Nauyuk Lake gives an estimate for the suspected mixed stock and at least confirms whether the published values are reasonable. The estimate for fish greater then 7 yr of age pooled over all years was 14.1%.

Using published values and the mixed-stock mortality value as guidelines, a natural mortality estimate of 15% was used.

Tagging mortality is also reported to be quite low. Berg and Berg (1990) reported no significant decrease in either survival or growth of tagged Arctic char larger than 36 cm. However, mortality of fish smaller than this was significant and was as high as 28.8% for smolts. Armstrong and Blackett (1966) reported less than 6% mortality of Dolly Varden (*Salvelinus malma*) tagged with comparable tags. Tagging mortality at Nauyuk Lake was also assumed to be low especially, since the fish were monitored for the first critical hours after tagging to ensure that recovery from the anesthetic was complete. An estimate of 3% was used, although this may be an underestimate for smaller fish.

The total difference between gross and net fidelity was estimated to be 20%.

Results

Estimated net fidelity of Arctic char in the Nauyuk Lake system appears to be quite low (Table 1). The mean net fidelity rate during the study was less than 55% with a maximum of 67% in 1978 and a minimum of 34% in 1974. The differences in annual fidelity rate were significant ($P <$

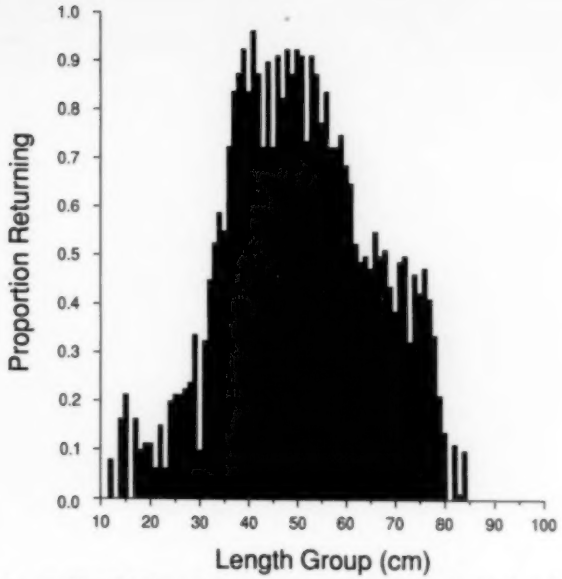

FIG. 2. Net fidelity of Arctic char to Nauyuk Lake by length group, 1974–78.

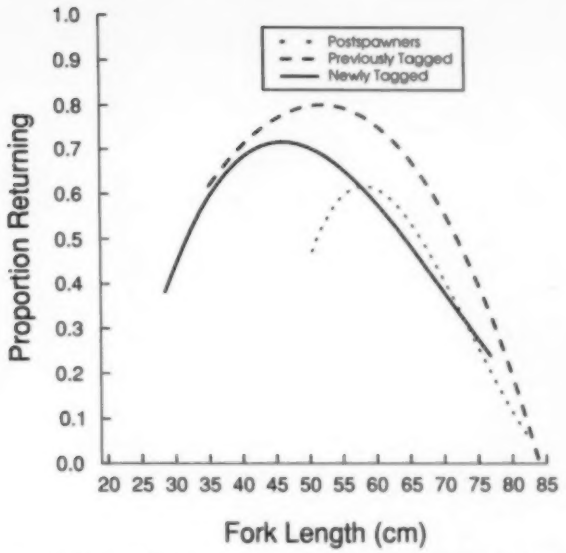

FIG. 3. Net fidelity of Arctic char to Nauyuk Lake as a function of length (third-degree polynomial).

0.003) when compared using ANOVA. While a number of char (7.2%) left the Nauyuk Lake system for 1 or 2 yr before returning, over 35% of the char that left in the spring never returned. Interestingly, the fidelity rate for fish returning in the same year generally increased from 1974 to 1978 whereas it decreased for fish returning after 1 or 2 yr of absence.

Fidelity was also size dependent. The ANOVA testing for significant differences in the proportion returning among size groups was highly significant ($P < 0.001$). Smaller and larger fish showed a much lower probability of returning than intermediate-sized fish (Fig. 2). However, this association is not a function of the size segregation of the downstream run. The regression of the day of downstream departure versus the probability of returning was not significant ($P < 0.26$). Similarly, the regression of the probability of returning versus the length of time at sea was also not significant ($P < 0.10$). Therefore, fidelity also does not seem to be related to length of time at sea.

The fidelity rates for the "postspawning", "previously tagged", and "newly tagged" groups of char are presented in Table 2. The fidelity rates for "newly tagged" and "postspawning" char are quite similar, while those for "previously tagged" char are notably higher. As all of the "postspawning" char were tagged and measured, annual differences and size-related differences are complete totals. The ANOVA comparing differences in the probability of returning among length classes yielded significant differences ($P < 0.001$) for both "newly tagged" and "previously tagged" groups, but the ANOVA comparing differences among years was only significant ($P < 0.005$) for "newly tagged" fish. There was no significant difference among years for "previously tagged" fish ($P = 0.157$). The ANOVA using both length group/years as blocks was highly significant ($P < 0.001$). However, this test was conservative and underestimated the actual relationship because the "newly tagged" group must have included Nauyuk char that had simply not been tagged before, as well as transient fish.

TABLE 2. Estimated net fidelity of Arctic char from Nauyuk Lake, 1974–78. Adjustments have been made for mortality and tag loss. N = number of char migrating downstream that were tagged; P_e = estimate of the proportion of char in the upstream run that migrated downstream in the spring; P_a = actual proportion of postspawning char that returned in the fall.

	Newly tagged		Previously tagged		Postspawners	
Year	N	P_e	N	P_e	N	P_a
1974	789	0.344	—	—	—	—
1975	698	0.405	377	0.670	32	0.625
1976	561	0.364	29	0.905	26	0.529
1977	1826	0.523	449	0.788	365	0.376
1978	414	0.538	610	0.760	216	0.561
Total	4288		1465		639	
Mean		0.451		0.748		0.458

The polynomial regressions calculated for each group are represented in Fig. 3. A third degree polynomial gave the highest correlation coefficient. The equations and correlation coefficients for each group are as follows:

"Postspawning":

$$\hat{F}_{net} = -14.943 + 0.683L + (-9.706 \times 10^{-3})L^2 + (4.395 \times 10^{-5})L^3;\ r^2 = 0.909$$

"Previously tagged":

$$\hat{F}_{net} = -0.614 + 0.046L + (-1.865 \times 10^{-4})L^2 + (-3.211 \times 10^{-6})L^3;\ r^2 = 0.881$$

"Newly tagged":

$$\hat{F}_{net} = -2.288 + 0.155L + (-2.487 \times 10^{-3})L^2 + (1.169 \times 10^{-5})L^3;\ r^2 = 0.928$$

where $\hat{F}_{net}$ = estimate of net fidelity and L = fork length (centimetres).

TABLE 3. Mortality estimates for Arctic char after leaving Willow Lake and before reaching the Nauyuk River. K = mean condition factor of char leaving Willow Lake.

Year	Number leaving Willow Lake	Number arriving at Nauyuk River	Mortality rate	K
1975	160	32	0.800	0.77
1976	194	26	0.866	0.67
1977	478	113	0.236	0.72
1978	258	216	0.163	0.73

Discussion

Fidelity to the Nauyuk Lake System

The data presented here suggest that 30–50% of the Arctic char that leave the Nauyuk Lake system each spring never return. However, the size of the Nauyuk run did not decrease by this amount. The decline that was observed in the size of the run during the study could largely be explained by known fishing mortality, overwintering mortality, and low recruitment (Johnson 1989; Gyselman and Broughton 1991). Therefore, char that left other systems in the spring apparently migrated into Nauyuk Lake in the fall compensating for infidelity losses. However, there seems to be two types of char leaving the system each spring: a high-fidelity group ("previously tagged") with a return rate of 70–90% and a low-fidelity group ("newly tagged") with a return rate of 35–55%. The high-fidelity group may be the true Nauyuk Lake stock, the fish that reproduce in the system. The low-fidelity group may be transients that are using the Nauyuk Lake system because of its easy accessibility. The return rate of the low-fidelity group is probably even lower than these estimates because the group must certainly have included high-fidelity fish that had not been tagged before.

The fidelity of the postspawning group was unexpectedly low in spite of the fact that, as spawners, these fish have the highest investment in the system. The mean for the postspawners was similar to that of the suspected itinerant "newly tagged" group (Table 2). However, a high mortality rate among postspawners may account for their apparent low fidelity in some years (Table 3). Mortality ranged from 16 to 86% between the time these fish left Willow Creek and the time they arrived at the Nauyuk River. This postspawning mortality appeared to decrease with time. However, the reason for this decrease is unknown. Postspawners leaving Willow Creek were also in much poorer condition (Table 3) than fish that overwintered in Nauyuk Lake (Gyselman and Broughton 1991). While the fish that died between Willow Creek and Nauyuk River were not included in the fidelity estimates, the postspawners that did make it to sea may have had a much higher mortality rate than the nonpostspawners.

Fidelity was not uniform among the length groups. Fidelity was highest in fish between 35 and 60 cm (Fig. 2), in some cases approaching almost 100%. However, fidelity decreased quickly beyond 65 cm. The decrease in fidelity with length has no easy explanation. The observed fidelity rate among fish of spawning size (60–80 cm) should have been higher if they have a strong affinity for their natal freshwater system. Even if mature char in this region spawn only every second or third year (Johnson 1989), the fidelity rate should have been relatively high. The reason for this low fidelity rate does not seem to be a higher mortality rate. A mortality estimate from the age–frequency data for fish age 10 and older which include only fish larger than 60 cm (Gyselman and Broughton 1991) was 15.2%, only slightly higher than for fish age 7 and older (14.1%). While this estimate is subject to the same limitations described above, there is no suggestion of a significant increase in mortality. In contrast, prespawning char that could presumably afford to "wander" until they reached maturity had a high fidelity rate. This paradox remains to be explained.

Fidelity was also much lower in smaller fish (Fig. 2), a phenomenon that was noted by Moore (1975). This may have been due to a higher mortality rate caused by physiological stress or higher predation rates. At sea, char less than 30 cm are susceptible to predation by various fish, marine mammals, and birds whereas those over 40 cm are relatively immune (Johnson 1980). Tagging mortality among smaller char may also have been much higher, a problem also noted by Berg and Berg (1990). Therefore, net fidelity may have been underestimated for these fish.

Size-related fidelity rates were observed in all three groups in the Nauyuk River run ("postspawned", "previously tagged", and "newly tagged" char). Figure 3 represents the fidelity of these groups. This model is of no biological significance. The third-degree polynomial equations that were used simply gave the best fit for the data and create a simpler visual representation. While the shape of the curves is very similar, the modal values are different. The modal value for the "previously tagged" fish may have been larger than that for the "newly tagged" group partly because the "previously tagged" fish had been tagged at least 1 yr earlier and, therefore, on average, were 1 yr older and correspondingly larger than the "newly tagged" fish. The modal size of the postspawning group is greater because only larger char spawn (Johnson 1989).

Management Implications

The results presented here do not support the present management assumption that each river system has a discrete stock, at least in the sense of Ricker's (1975) definition. If the Nauyuk Lake system is typical, then there is the possibility of a significant interchange of char among river systems. There are other data supporting this hypothesis. Dempson and Kristofferson (1978) have also shown that char move among systems, although their estimates of the rate of exchange were not as high as those given here. Johnson (1980) reported that the number of char migrating up the Diana River, Northwest Territories, nearly doubled over a 2-yr period. This increase could not be explained by recruitment from the stock. Similarly, Armstrong (1984) reported that Dolly Varden in Alaska may use streams other than their natal streams to overwinter in nonspawning years. In these latter two cases, there were yearly fluctuations in the size of the runs. In contrast, the size of the run in the Nauyuk River never increased during successive years. The run always declined. A significant proportion of this decline was due to Inuit harvesting and very low recruitment (Gyselman and Broughton 1991), but still, there was no direct evidence of immigration of char from other systems at levels greater than the estimated emigration rate of Nauyuk Lake fish. The reason for this apparent balance of emigration and immigration is unknown. However, it does emphasize the

apparent complexity of the migration dynamics of anadromous Arctic char populations.

Nauyuk Lake char are known to emigrate both for relatively short periods, as shown by the number of fish returning after 1 or 2 yr (Table 1), and for indefinite periods, as demonstrated by tag returns from other systems, some relatively close (20–30 km) but others as far away as 550 km (Gyselman 1984). The number of tag returns from other systems was small (1% of the tags applied), but fishing pressure on most river systems in the area is light. However, it is significant that char tagged at Nauyuk Lake have been returned from virtually every system within a 600-km range that has either a domestic or commercial fishery.

The fidelity data presented here also suggest that significant immigration takes place. Unfortunately, no large-scale tagging programs were carried out on other nearby rivers. Therefore, while the evidence supports the theory that there could be a significant exchange of char between the Nauyuk Lake system and other river systems, it could not be directly measured. This apparent infidelity of char does not preclude that each river system may be a discrete genetic stock. Little genetics work has been published on char in the Canadian Arctic, although studies are currently being done. The results of these studies will be extremely valuable to the understanding of the population structure of char. However, Reist (1989) concluded that Dolly Varden in a number of rivers on the north slope of the Yukon were true genetic stocks even though they were known to mix at sea.

Char in the central Canadian Arctic only spawn every 2–4 yr (Johnson 1980) and presumably could overwinter and feed in other systems before returning to Nauyuk Lake to spawn. At Nauyuk Lake, char that were known to have left the system for 1 or 2 yr were observed migrating up Willow Creek to spawn in the spring following their return. However, no clear indication could be made as to whether or not these fish returned specifically to spawn.

This studies raises many questions about how to approach the analysis of the population dynamics data and the subsequent management of Arctic char stocks. The degree of exchange among river systems is probably spatially and temporally variable. Certainly the proximity of suitable rivers to one another may be important. Climate could also be very influential. For example, after dry summers, smaller rivers have been observed to become impassable, presumably forcing the stock that migrated downstream in the spring to use another river to overwinter. Not only does weather affect the originating stream in this case but also the dynamics of the recipient river that must accept immigrants.

Arctic char are probably a good example of a "metapopulation". Hanski and Gilpin (1991, p. 7) defined a metapopulation as "a set of local populations which interact via individuals moving among populations". This is a somewhat more liberal and practical definition than the original put forward by Levins (1969). Metapopulation dynamics introduces another level of structure to explain the exchange of individuals among populations. This exchange of individuals and their plasticity in morphology is what make char notoriously "good colonizers". Exchange of individuals also permits recolonization of unstable river systems. These features allow char populations to maintain relative stability in an unpredictable environment.

If the fidelity rates and migration distances observed in Nauyuk Lake are indicative of the general behaviour of char throughout the Canadian Arctic, then management becomes considerably more difficult than is now generally acknowledged. Dempson and Kristofferson (1987) have proposed the idea of "local stock complexes" which recognizes that a fishery may be harvesting fish from a number of river systems. However, for many fisheries, it is still unknown whether each river has a discrete genetic stock. Determining whether this is true is a logical first step in developing a management approach for char fisheries. If each river has a discrete genetic stock that can be identified, then the composition of the catch from the fishery at different geographic locations can be determined. This then leads to the use of the multi-stock management model. On the other hand, if each river does not have a genetically discrete stock, then a zone-management plan is a logical approach. However, the problem then becomes defining the extent of the zone. In the future, the development of suitable metapopulation models may eventually lead to a better strategy for char management.

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Seasonal Changes in the Total Lipid Content of the Opossum Shrimp, *Mysis relicta* (Malacostraca: Mysidacea)

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Adare, K.I., and D.C. Lasenby. 1994. Seasonal changes in the total lipid content of the opossum shrimp, *Mysis relicta* (Malacostraca: Mysidacea). Can. J. Fish. Aquat. Sci. 51: 1935–1941.

Seasonal changes in the percent total lipid content of *Mysis relicta* were investigated in four lakes in southern Ontario. Stony and Twelve Mile lakes contain populations of *Mysis* with a 1-yr life cycle, while Crystal and Boshkung lakes have populations with a 2-yr life cycle. Mature female and male mysids have a lipid content between 15 and 33% and between 14 and 25%, respectively, while juvenile mysids have approximately 10% less total lipids than adults. The relationship between total lipid and lipid-free dry weight is best described by the power curve $Y = aX^b$ and is the same (ANCOVA, $p < 0.05$) in all four mysid populations. Lipid content accumulates steadily with time as the mysid grows, and lipid reserves do not appear to influence mysid time to maturity. For studies on the transfer of energy and lipophilic organic contaminants through the food web, the results imply that seasonal and body size differences of organisms are important considerations.

Les variations saisonnières de la teneur en lipides totaux chez *Mysis relicta* ont été étudiées dans quatre lacs dans le sud de l'Ontario. Les lacs Stony et Twelve Mile renferment des populations de *Mysis* dont le cycle vital est de 1 an, alors que les lacs Crystal et Boshkung renferment des populations dont le cycle vital est de 2 ans. Les mysidacés parvenus à maturité ont une teneur en lipides qui varie entre 15 et 33 % chez les femelles et entre 14 et 25 % chez les mâles, alors que les juvéniles ont une teneur en lipides totaux environ 10 % inférieure à celle des adultes. La meilleure courbe de puissance pour décrire la relation entre les lipides totaux et le poids sec sans les lipides est $Y = aX^b$, et c'est la même (ANCOVA, $p < 0.05$) chez les quatre populations de mysidacés. Les lipides s'accumulent régulièrement avec la croissance des mysidacés et les réserves lipidiques ne semblent pas influencer le temps que prend le mysidacé pour parvenir à maturité. En ce qui concerne les études sur le transfert d'énergie et les contaminants organiques lipophiles dans la chaîne alimentaire, les résultats signifient que les différences saisonnières et les variations de la taille des organismes sont des facteurs importants.

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The lipid content of freshwater zooplankters influences both their reproduction and survival (Goulden and Henry 1984). As an energy reserve, lipids have more than twice as many calories per unit dry weight as proteins or carbohydrates (Tessier et al. 1983; Quigley et al. 1989). Measuring changes in lipid content in zooplankton is critical, therefore, to understanding both life cycle characteristics of individual zooplankton species and energy transfer in food webs. In addition to being an essential physiological component of zooplankton, lipids harbour organic contaminants (Harding 1986), and seasonal changes in lipid content within an organism may affect the transfer and partitioning of lipophilic contaminants in aquatic systems (Evans et al. 1982).

The lipid content of freshwater zooplankton can be affected by season, food availability, and life cycle stage (Tessier and Goulden 1982; Hakanson 1984; Cavaletto et al. 1989; Gauvin et al. 1989; Arts and Evans 1991; Hill et al. 1992; Vanderploeg et al. 1992). Generally, juvenile zooplankton have lower lipid content than adults, and females have a higher lipid content than males. As well, lipid content in crustacean zooplankton species accumulates over time (larger, older animals have a proportionally higher lipid content than smaller, younger animals). However, the relationship between lipid content and the duration of an organism's life cycle has yet to be investigated.

The purpose of this study was to describe the life cycle characteristics and quantify the total lipid content of four populations of the freshwater crustacean *Mysis relicta*. Two of the populations have 1-yr life cycles (time to maturity) and two have 2-yr life cycles (D.C. Lasenby, unpublished).

Methods

Mysid populations were sampled bimonthly from May to November 1990 from four lakes located in southern Ontario. The lakes ranged in trophic status from mesotrophic Stony (78°20′W, 44°40′N) and Crystal (78°19′W, 44°45′N) lakes to oligotrophic Twelve Mile (78°42′W, 45°01′N) and Boshkung (78°44′W, 45°04′N) lakes (Dillon 1974; OMOE 1978, 1979; K.I. Adare, unpublished).

A minimum of 100 mysids were collected from Crystal, Stony, and Boshkung lakes approximately bimonthly for lipid analysis and to determine the life cycle characteristics of the populations. However, in Twelve Mile Lake, due to a low population density, less than 100 mysids were often caught. Within 12 h of capture, the lengths of five or six males, five or six females, and 15–25 juveniles were determined and the

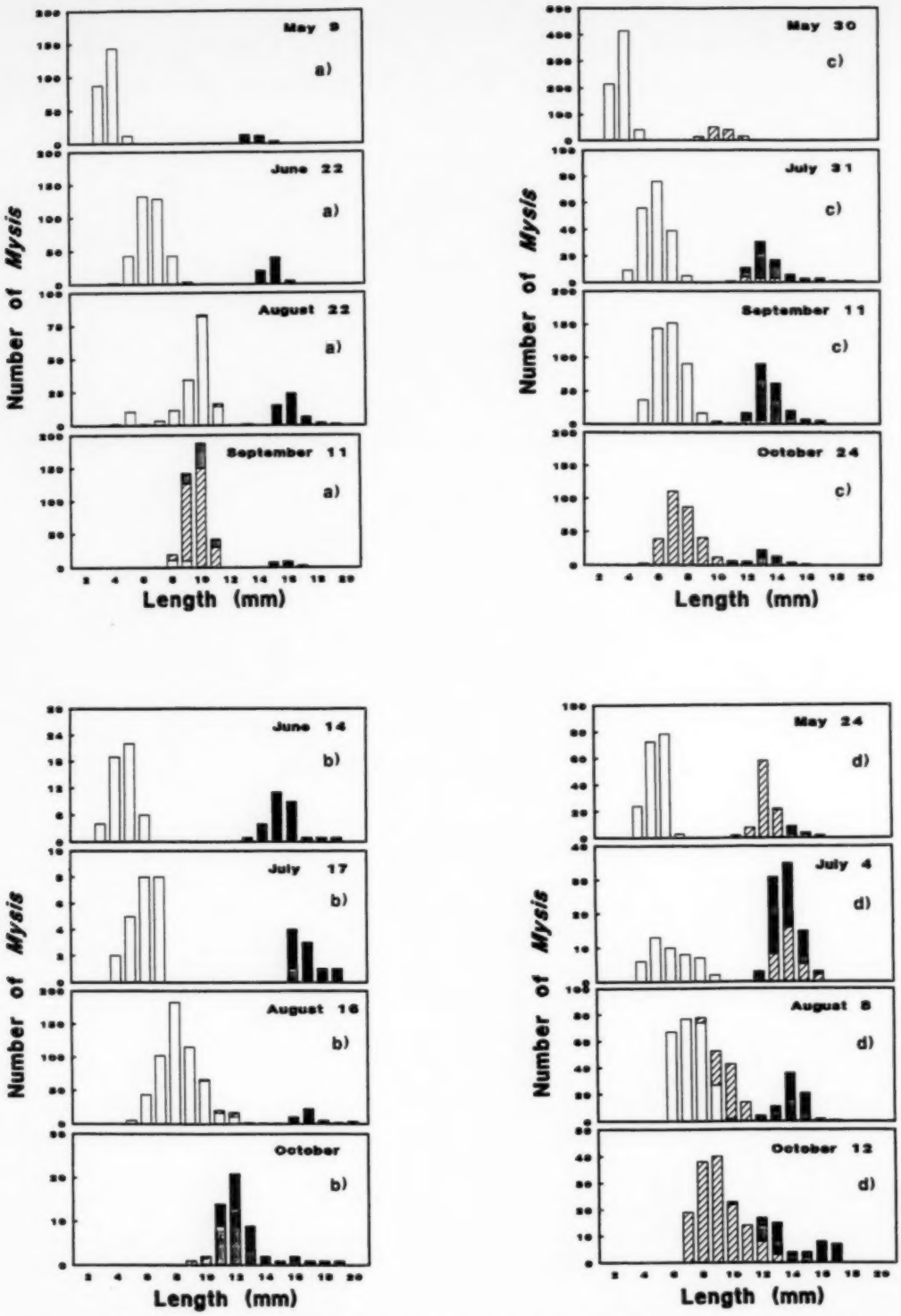

FIG. 1. Size–frequency distributions of *M. relicta* in (a) Stony, (b) Twelve Mile, (c) Crystal, and (d) Boshkung lakes. Open bars represent juveniles, solid bars represent females, hatched bars represent immatures, and shaded bars represent males.

TABLE 1. Lake trophic status, life cycle, growth rate, average length, lipid content, and percent lipid of *M. relicta* from the four study lakes after 6 (upper value) and 18 mo (lower value) of growth.

Lake	Trophic status	Life cycle	Growth rate ($mm \cdot mo^{-1}$)	Mean length (mm)	Lipid (mg) ± SE (n)	% lipid ± SE
Stony	Mesotrophic	1 yr	1.5	14.4	1.626 ± 0.107 (99)[a]	22 ± 0.06[b]
			0.5	16.0	2.052 ± 0.266 (5)	29 ± 4.77
Crystal	Mesotrophic	2 yr	0.7	8.2	0.116 ± 0.011 (4)	11 ± 0.94
			0.5	13.0	1.464 ± 0.149 (11)	25 ± 2.12
Twelve Mile	Oligotrophic	1 yr	1.8	14.5	1.250 ± 0.151 (6)	20 ± 1.27
			0.5	16.0	2.259 ± 0.256 (4)	27 ± 3.75
Boshkung	Oligotrophic	2 yr	1.0	10.0	0.435 ± 0.024 (3)	27 ± 1.44
			0.4	14.0	1.362 ± 0.194 (5)	29 ± 3.92

[a]Predicted using the relationship $Y = 3.7 \times 10^{-5} X^{4.02}$ ($R^2 = 0.99$) where Y is lipid (mg) and X is length (mm).

[b]Predicted using the relationship $Y_1 = -0.22 + 1.56x$ ($R^2 = 0.60$) where Y_1 is % lipid and x is length (mm).

animals frozen for future lipid analysis. Males were not recognizable in the spring, as they do not develop secondary sexual characteristics until fall. Three or four individual juveniles were pooled as one sample to ensure a biomass of greater than 1.0 mg dry weight necessary for lipid analysis (Gardner et al. 1985a).

Total lipids were quantified by the chloroform–methanol extraction method for aquatic invertebrates described by Gardner et al. (1985a). To minimize the breakdown of lipids during sample storage (Christie 1982), 0.5 mL of HPLC-grade chloroform was added to each mysid sample and the samples were stored in gas-tight scintillation vials at −20°C. Prior to lipid extraction, the chloroform was evaporated from the frozen samples by drying at 50°C for 24 h in a nitrogen atmosphere. Mysid dry weight and the final weight of the lipid extract were determined on a Mettler balance (±1 μg).

The results of the lipid analysis are expressed either as total lipid content, which reflects the amount (milligrams) of lipid per individual, or as percent lipid, which is calculated as the lipid content divided by the lipid-free dry weight (LFDW) of the individual. LFDW is calculated as the dry weight of the animal minus the total lipid content.

The remaining mysids were preserved in 75% ethanol for life cycle determinations. Length was measured from the tip of the rostrum to the apical cleft of the telson. A male was considered mature if the fourth pleopod extended beyond the telson; females were distinguishable by the presence of a marsupium. Individuals with no sexual characteristics were classified as juveniles.

Samples collected for lipid analysis from Stony, Twelve Mile, and Boshkung lakes in late November 1990 included gravid females. The eggs in the marsupium were removed from these females to eliminate the lipid fraction in the embryos. We attempted to determine the lipid content of the eggs but found them too fragile to transfer for analysis. To compare reproductive effort between the populations, we collected gravid females in the fall of 1991 and counted the number of eggs per brood.

A minimum percent lipid content for mysids was determined in the laboratory and compared with percent lipid content of natural populations of mysids to determine if mysids experience starvation conditions in the lake. Approximately 40 adult *M. relicta* were collected from Stony Lake on May 9, 1990, and held in a tank at 4°C to determine the rate of decline of lipid to a minimum percent lipid. The mysids were not fed, although it is possible that dead mysids were scavenged or cannibalized. Five to eight mysids were sampled approximately weekly for 45 d and their percent lipid determined.

TABLE 2. Relationship between mysid lipid content (Y) and LFDW (X) for the four study lakes; $Y = aX^b$.

Lake	n	b	a	R^2
Stony	99	1.411	0.128	0.95
Crystal	97	1.222	0.150	0.97
Twelve Mile	96	1.310	0.099	0.96
Boshkung	147	1.400	0.144	0.96

Results

A cohort of juvenile mysids (mean length approximately 3.5 mm) was present in the spring in all four study populations of mysids. In Stony (Fig. 1a) and Twelve Mile (Fig. 1b) lakes, this cohort grew at rates of 1.5 and 1.8 $mm \cdot mo^{-1}$, respectively (Table 1). The cohort of juveniles in Crystal (Fig. 1c) and Boshkung (Fig. 1d) lakes, however, grew at slower rates of 0.7 and 1.0 $mm \cdot mo^{-1}$, respectively (Table 1). All four populations of mysids also had a second cohort of larger animals that grew at approximately 0.5 $mm \cdot mo^{-1}$. In Stony (Fig. 1a) and Twelve Mile (Fig. 1b) lakes the larger cohort (15 mm) comprised all adult females. In Crystal (Fig. 1c) and Boshkung (Fig. 1d) lakes the second cohorts (10 mm) were mostly immature mysids, although several females were present in the Boshkung Lake cohort (Fig. 1d). After approximately 6 mo, in all populations the two cohorts were not as distinct and consisted of immature, male, and female individuals. Gravid females were collected from all four mysid populations in November.

In all four study populations, the relationship between body size (LFDW) and lipid content is best described by the power curve $Y = aX^b$ (Table 2). Lipid content, as described by LFDW, was not significantly different (ANCOVA, $p < 0.05$) between male and female mysids in Stony, Twelve Mile, and Boshkung lakes; therefore, the results for males and

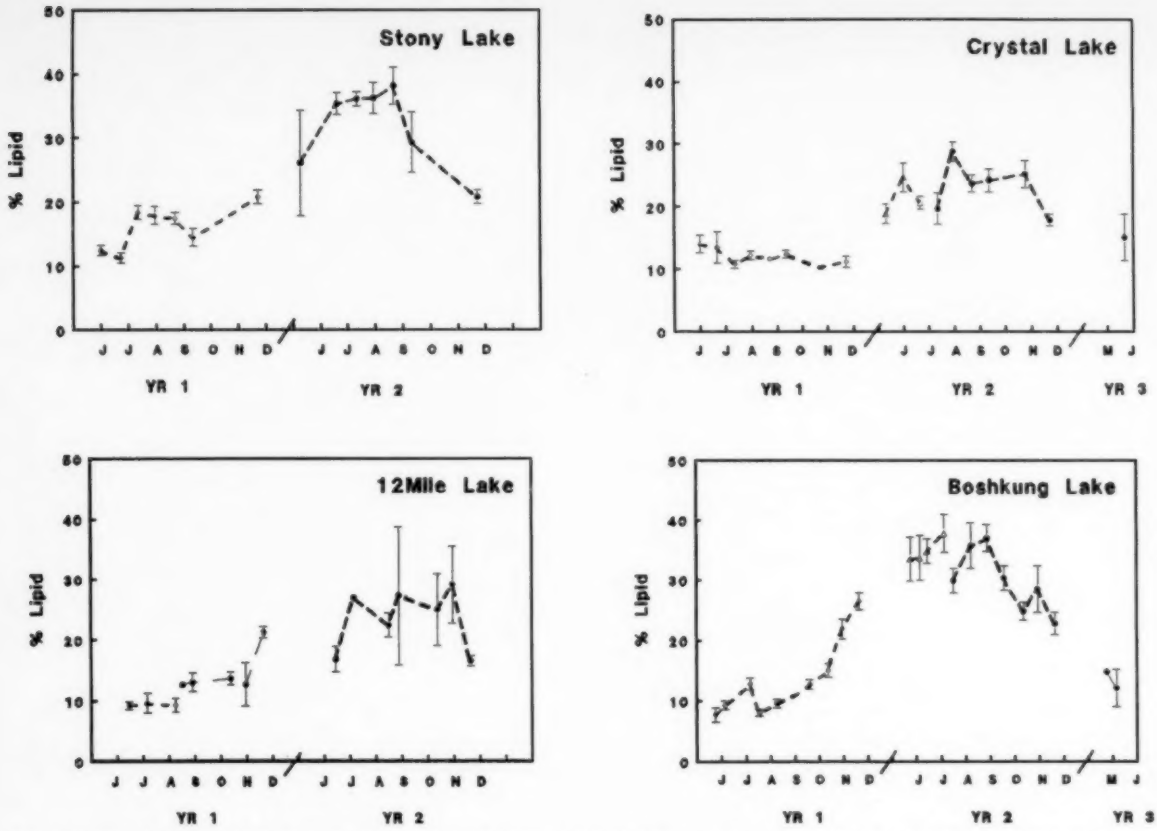

FIG. 2. Seasonal changes in the percent lipid of juvenile (open circles), immature (open triangles), and mature (solid circles) mysids from May to November 1990 in the four study lakes. Error bars represent ±1 SE; data are presented over the lifespan of the populations for ease of illustration.

TABLE 3. Mean, standard error, and number of eggs·brood^{-1} in gravid females collected in November 1991 from Stony, Crystal, and Boshkung lakes.

Lake	*n*	Mean[a]	SE
Stony	23	17.7	0.78
Crystal	25	15.5	0.65
Boshkung	18	16.8	0.78

[a]No significant differences (ANCOVA, $p < 0.05$).

females were pooled and expressed as adults. There was a significant difference in lipid content between male and female mysids in Crystal Lake (ANCOVA, $p < 0.05$); however, the samples were pooled, as the probability of there being no difference in lipid content between males and females ($p = 0.03$) was close to the chosen level of significance ($\alpha = 0.05$). The rate of increase of lipid with dry weight was not significantly different between the four lakes, and the lipid content was similar in all four lakes for mysids of similar size (ANCOVA, $p < 0.05$). The data were log-transformed to meet the test assumptions of homogeneity of variance and normality of residuals.

Mysis relicta from 2-yr life cycle populations accumulated much less lipid in 6 mo than those from populations with a 1-yr life cycle (Table 1). At maturity, mysids from a 2-yr life cycle have less total lipid but a similar percent lipid to mysids from a 1-yr life cycle. Because there were no mysids caught from Stony Lake in November, we estimated the length of a 6-mo-old animal from the growth rate over the summer months (1.5 mm·mo^{-1}). The predicted length is similar to that obtained by Lasenby and Langford (1972) for Stony Lake mysids. We used this value to predict lipid content and percent lipid based on length–lipid content regressions.

Lipid levels ranged from 9 to 40% and followed similar seasonal trends in mysids from all four populations (Fig. 2). Percent lipid in adult animals was similar in late fall and early spring, reached a peak between July and the end of August, and slowly declined until November. Adult mysids accumulated the largest proportion of lipid in Boshkung and Stony lakes, 40 and 38%, respectively, and only up to 30% in Crystal and Twelve Mile lakes. Juvenile mysids in all four lakes had approximately 10% less lipid than adults.

There was no relationship ($n = 66$, $R^2 = 0.03$) between female length and number of eggs per brood, and there was no difference in the number of eggs per brood (ANCOVA, $p < 0.05$) among Stony, Crystal, and Boshkung lakes (Table 3); only one gravid female was collected from Twelve Mile Lake.

TABLE 4. Summary of published estimates of ranges in percent lipid (based on dry weight), feeding type, and habitat in some freshwater invertebrate taxa.

Organism	% lipid range	Length of study (mo)	Feeding type	Habitat	Source
Copepoda					
Diaptomus sicilis	14–42	16	Herbivore	Pelagic	Vanderploeg et al. 1992
Limnocalanus macrurus	42–67 (♀)	5	Herbivore	Pelagic	Cavaletto et al. 1989
Senecella calanoides	29	5	Herbivore	Pelagic	Cavaletto et al. 1989
Cladocera					
Daphnia pulex	20–31	Laboratory study	Herbivore	Pelagic	Goulden and Place 1990
Daphnia magna	17–22	Laboratory study	Herbivore	Pelagic	Goulden and Place 1990
Amphipoda					
Pontoporeia hoyi[a]	28–41	8	Herbivore/ detritivore	Benthic	Gauvin et al. 1989
P. hoyi	17–47	10	Herbivore/ detritivore	Benthic	Gardner et al. 1985b
P. hoyi	30 (♀) 10 (♂) 21 (juv.)	1	Herbivore/ detritivore	Benthic	Quigley et al. 1989
Monoporeia affinis	13–27	11	Herbivore/ detritivore	Benthic	Hill et al. 1992
Pontoporeia femorata	17–23	11	Herbivore/ detritivore	Benthic	Hill et al. 1992
Zooplankton community	8–24	10	Herbivore	Pelagic	Wainman and Lean 1989
Ologochaeta					
Lumbriculid					
Stylodrilus veringianus	12–19	10	Detritivore	Benthic	Gardner et al. 1985b
Tubificid	9–22	7	Detritivore	Benthic	Gardner et al. 1985b
Insect larvae					
Chironomidae	9–39	5	Herbivore/ detritivore	Benthic	Gardner et al. 1985b
Mysids					
Mysis relicta	26–37	4	Omnivore	Pelagic/ benthic	Gardner et al. 1985b
M. relicta	10–41	7	Omnivore	Pelagic/ benthic	This study

[a]Recently renamed *Diporeia hoyi* (Bousfield 1989).

In the laboratory starvation experiment the percent lipid of the Stony Lake mysids (initially 30%) declined to a minimum of approximately 7% after 35 d and remained stable to 45 d. This value is much lower than that observed in adults from the study populations. However, 6-mo-old Crystal Lake mysids had 11% lipids (Fig. 2), which approached the 7% starvation value observed in the laboratory.

Discussion

The size–frequency histograms and calculated growth rates confirm that mysids from Stony and Twelve Mile lakes have 1-yr life cycles, while mysids from Crystal and Boshkung lakes have 2-yr cycles. The presence of a cohort of large mature females (15 mm) in both Stony (Fig. 1a) and Twelve Mile (Fig. 1b) lakes indicates that populations of mysids in these lakes live up to 2 yr and reproduce in both their first and second year. This observation is similar to those for other mysid populations with 1-yr life cycles (Lasenby and Langford 1972; Morgan and Beeton 1978; Morgan 1981).

Juvenile mysids have approximately 10% less lipid than adults in all four mysid populations. A similar difference was reported between adult female and juveniles of the deposit-feeding amphipods *Pontoporeia hoyi* (Quigley et al. 1989) and *Monoporeia affinis* and *P. femorata* (Hill et al. 1992). Goulden and Hornig (1980) suggested that juvenile planktonic cladocerans are less likely to store energy as lipids due to the higher energy demands for growth. The lack of energy storage by juvenile cladocerans may also apply to mysids, as the growth rates of juvenile mysids (0.7–1.8 $mm \cdot mo^{-1}$) were greater than those of adult mysids (0.4–0.5 $mm \cdot mo^{-1}$) in all four populations. Ouellet et al. (1992) found low absolute amounts of triacylglyceride, a storage lipid common to mysids (B. Wainman, unpublished), in the larvae of the shrimp *Pandalus borealis* reared at various food conditions, indicating the priority of development and growth over the accumulation of energy reserves during juvenile development.

Percent lipid (10–41%) in *M. relicta* from the four study lakes is within the range of reported values for other aquatic invertebrates including mysids (Table 4). Lipids range from 14 to 67% of dry weight for pelagic zooplankton and from

9 to 54% for benthic organisms, depending on the species and the environment. Gardner et al. (1985b) found 19–37% lipids in *M. relicta* from Lake Michigan, similar to that found in our study. However, comparison of seasonal changes in percent lipid of Lake Michigan *M. relicta* with our study is limited because Gardner et al. (1985b) collected infrequently and the size of the mysids analyzed is not given.

In our study, percent lipid varied depending on the size of the animal, the season, and the lake (Table 1). The power curve relationship between body size (LFDW) and lipid content for *M. relicta* may be attributed to the fact that larger body sizes provide an energetic advantage with respect to food intake minus metabolic costs (Brooks and Dodson 1965), at least until the end of their second season (see Fig. 2). Also, as mysids become larger, they are known to shift part of their diet from phytoplankton to zooplankton which may also represent a shift to a higher food quality.

In all four study populations, lipid levels reach a peak between July and the end of August during the second season of growth and then slowly decline from August until November (Fig. 2), suggesting that these 2-yr-old mysids are decreasing their food intake. Seasonal fluctuations of percent lipid in *M. relicta* does not appear to conform to patterns of accumulation and loss of lipid energy reserves in other freshwater zooplankton. Although there are slight seasonal fluctuations in the percent lipid of mysids (Fig. 2), the general trend is one of accumulation of lipid through time. However, cladocerans and copepods, common pelagic zooplankton species, inhabit environments in which regular periods of low food availability stress the populations (Stearns 1976), and they demonstrate considerable temporal variation throughout their life cycle in the quantity of lipid stored (Tessier and Goulden 1982; Arts and Evans 1991; Vanderploeg et al. 1992). As well, the amphipods *P. hoyi* and *P. femorata* demonstrate a pattern, similar to cladocerans and copepods, which characterizes an organism that increases its energy reserves during periods of high food availability (i.e., during spring diatom bloom) and utilizes the lipid resources during periods of low food availability (Gauvin et al. 1989; Hill et al. 1992).

The lack of high seasonal fluctuations of lipids in mysids throughout their life cycle may be a result of the omnivorous feeding habits of *M. relicta* (Grossnickle 1982; Lasenby et al. 1986). *Mysis relicta* exhibits diel vertical migration, remaining on the bottom of the lake during the day and migrating into the water column at dusk (Bowers 1988; Levy 1991). Mysids may be less susceptible to periods of starvation for two reasons. Because of their omnivory, they do not depend solely on the fluctuating biomass of the plankton community as a primary food supply, and their diel vertical migratory behaviour exposes them to benthic (Parker 1980) as well as pelagic (Lehman et al. 1990) food resources.

Because it is directly affected by food intake (Cowgill et al. 1984; Fraser 1989; Goulden and Place 1990; Ouellet et al. 1992), lipid content might be expected to be greater in mysids from a eutrophic lake than from an oligotrophic lake as described for the copepod *Diaptomus sicilis* (Arts and Evans 1991) and brook trout, *Salvelinus fontinalis* (Nelson and McPherson 1987). However, lipid content of the four populations of *M. relicta* studied does not reflect lake trophic status. The lipid content accumulated by a 6-mo-old mysid from oligotrophic Twelve Mile Lake (1.250 ± 0.151 mg) is similar to that accumulated by an individual from mesotrophic Stony Lake (1.626 ± 0.107 mg) (Table 1). Increased lake trophic status may imply an increase in phytoplankton biomass, but it may not necessarily result in an increase in the quality or quantity of food for a mysid population.

Hill et al. (1992) suggested that if amphipods are unable to obtain enough food in their first season to grow large enough to store lipid to a level of approximately 20% of their dry weight, they may have to postpone reproduction until the following year. However, for *M. relicta*, it appears that body size and total amount of lipid accumulated rather than percent lipid were more important for reproduction. Boshkung Lake mysids, which reproduce after 18 mo, have a similar percent lipid (27%) but smaller body size after 6 mo than Stony Lake and Twelve Mile Lake mysids which reproduced in 6 mo (Table 1). Boshkung Lake mysids do not reproduce until they have accumulated 1.36 mg of lipid despite a high percent lipid after 6 mo. As well, Crystal Lake mysids reproduce after 18 mo when their lipid content is 1.46 mg. This population may be food limited in their first year, resulting in both low total lipid content and slow growth rates. The low percent lipid of the Crystal Lake mysids (11%) after their first 6 mo of growth was close to the percent lipid of starved mysids (7%), suggesting that food resources were not as available to this population as to the other three populations. However, by the end of their second season, the lipid content and percent lipid of adult Crystal Lake animals are similar to those of the other three populations, reflecting a possible shift in food resources as the animals mature.

The percent lipid of adult mysids in all four study populations was never as low as the 7% minimum percent lipid observed in the laboratory, indicating that adult mysids in the field do not experience extreme starvation. In our experiment, after 45 d, lipid droplets, normally visible under the animals' carapace, were absent, indicating that starved mysids can use this energy reserve.

Zooplankton use accumulated storage lipids for egg synthesis (Cowgill et al. 1984; Goulden and Henry 1984). Reproductive effort has been investigated in several populations of *M. relicta* with varying life cycles (Lasenby and Langford 1972). Beeton and Gannon (1991) found a positive relationship between the number of eggs per brood and the length of gravid females and a correlation between lake productivity and brood size. Because there was no significant difference in lipid content of mysids at maturity among the four populations (Table 1), it was expected that their reproductive effort, as measured by the number of eggs per brood, would be similar (Table 3). In our study, there is no correlation between brood size and lake productivity. It is possible that the lakes in Beeton and Gannon's (1991) study encompassed a greater range of productivity than the lakes in this study.

For mysids, reproduction appears to depend on the population achieving a threshold body size and lipid content. Lipids in mysids gradually accumulate over time, and the rate of increase is dependent on body size regardless of life cycle duration (Table 2). Energy reserves in *M. relicta*, as measured by percent lipid, do not reflect or appear to influence time to maturity. Mysids can store energy as lipid and will use these reserves during periods of extreme artificial starvation; however, because of their feeding and migratory behaviour, they are unlikely to experience such extreme conditions in the lake environment.

Mysis relicta plays a significant role in the cycling of energy and contaminants in lake ecosystems because it interacts with both benthic and pelagic zones in lakes and because it is a prime food item for many pelagic predators (Van Duyn-Henderson and Lasenby 1986; Val Klump et al. 1991). Increases in lipid content in mysids and other zooplankton, due to differences in either size or seasonality of the organism, may increase organic contaminant concentrations (Harding 1986) and energy content (Tessier et al. 1983; Quigley et al. 1989) and significantly affect the availability and transfer of energy and/or contaminants through the food chain.

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Otolith Elemental Fingerprinting for Stock Identification of Atlantic Cod (*Gadus morhua*) Using Laser Ablation ICPMS

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Campana, S.E., A.J. Fowler, and C.M. Jones. 1994. Otolith elemental fingerprinting for stock identification of Atlantic cod (*Gadus morhua*) using laser ablation ICPMS. Can. J. Fish. Aquat. Sci. 51: 1942–1950.

Trace element incorporation into fish otoliths varies among samples collected at different sites. If otolith elemental composition (the elemental "fingerprint") somehow reflects the characteristics of the ambient water, the elemental fingerprint of the otolith nucleus could serve as a natural marker of fish hatched at different sites. To test this hypothesis, Atlantic cod (*Gadus morhua*) otoliths collected from five spawning grounds in the northwest Atlantic were tested for differences in elemental and isotopic composition. Laser ablation – inductively coupled plasma mass spectroscopy (LA-ICPMS) was used to assay the concentration of 14 isotopes (nine elements) in otolith nuclei. The sensitivity of the laser ablation system exceeded that of the electron microprobe by 2–4 orders of magnitude, with an average CV of 21% for any given isotope. Most isotopic concentrations were consistent between left and right otoliths of a given fish, and most differed significantly among sample sites; there were no significant differences by age, sex, or fish length. Multivariate analyses of the elemental fingerprints resulted in significant discrimination among sample sites. While the mechanism underlying trace element incorporation into otoliths is still unclear, otolith elemental fingerprinting has the potential to become an effective and accurate means of stock identification.

L'incorporation d'éléments traces dans les otholites des poissons varie dans des échantillons prélevés à différents endroits. Si la composition élémentaire des otolithes reflétait de quelque manière les caractéristiques de l'eau dans laquelle évolue le poisson, cette composition (la composition élémentaire du noyau des otolithes) pourrait servir à marquer naturellement les poissons nés à différents endroits. Dans le but de vérifier cette hypothèse, des otolithes de morue franche (*Gadus morhua*) prélevés de cinq frayères dans le nord-ouest de l'Atlantique ont fait l'objet de tests visant à déceler des différences dans la composition élémentaire et isotopique. On a utilisé l'ablation par laser couplée à la spectroscopie d'émission avec plasma induit par haute fréquence pour déterminer la concentration de 14 isotopes (neuf éléments) dans des noyaux d'otolithe. La sensibilité du système d'ablation par laser était de 2 à 4 fois supérieure à celle de la sonde électronique, avec un CV moyen de 21 % pour chaque isotope. La plupart du temps, la concentration des isotopes était comparable dans les otolithes gauche et droit d'un poisson, et elle variait significativement d'un endroit échantillonné à l'autre; on n'a observé aucune différence significative en fonction de l'âge, du sexe ou de la longueur du poisson. Une analyse multivariée de la composition élémentaire a révélé des différences importantes entre les endroits d'échantillonnage. Le mécanisme responsable de l'incorporation des éléments traces dans les otolithes est encore peu connu, mais la composition élémentaire des otolithes pourrait devenir un moyen efficace et précis d'identification des stocks.

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Recent studies have suggested that the elemental composition, or "elemental fingerprint", of the fish otolith may prove to be among the most powerful means yet developed for distinguishing among fish stocks (Edmonds et al. 1989, 1991, 1992; Kalish 1990; Gunn et al. 1992; Campana and Gagné 1994). The success of the approach is based on two observations: (1) otoliths grow throughout the life of the fish and, unlike bone, are metabolically inert; once deposited, otolith material is unlikely to be resorbed or altered (Campana and Neilson 1985; Casselman 1987); and (2) the calcium carbonate and trace elements that make up 90% of the otolith appear to be mainly derived from the water (Simkiss 1974). Accordingly, the elemental composition of the otolith may reflect that of the water in which the fish lives, although not necessarily in a simplistic fashion (Kalish 1989). Since the elemental composition of seawater varies from place to place (Johnson et al. 1992), the elemental composition of the accreting otolith may vary with the water mass in which the fish is swimming. By corollary, the composition of the otolith nucleus, corresponding to the otolith around the time of hatch, would reflect the composition of the water at the hatch (spawning) site, and fish spawned at different sites would contain otolith nuclei that vary in their composition.

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TABLE 1. Sample collection information. Each sample consisted of 30 fish.

Sample	Area	NAFO division	Collection date	Mean length (range), cm	Mean age (range), yr
1	Cheticamp	4T	May 26, 1986	50.5 (45–63)	5.9 (5–8)
2	Fundyrip	4X	Mar. 6, 1986	54.5 (45–74)	3.1 (2–4)
3	Georges Bank	5Zj	Apr. 16, 1986	62.1 (48–77)	3.9 (2–6)
4	Iceland	—	May 6, 1986	70.1 (62–75)	6.8 (5–10)
5	Newfoundland	30de	May 26–28, 1986	62.4 (52–69)	6.9 (5–9)

Otolith elemental composition has been used to infer stock identity both through analysis of dissolved whole otoliths and through use of beam probes. The former approach is based on the assumption that the geographic ranges of populations differ, at least on average. Given elemental differences in water composition between stock home ranges, and assuming that otolith composition reflects that of water, otolith elemental composition should also differ. Site-specific differences in elemental concentrations based on analysis of dissolved whole otoliths have been reported by a number of workers (Papadopoulou et al. 1978, 1980; Edmonds et al. 1989, 1991, 1992; Grady et al. 1989; Campana and Gagné 1994), despite the likelihood of significant ontogenetic effects (Grady et al. 1989). In general, stock discrimination capabilities have improved with the sensitivity of the instrumentation, reflecting the relative purity of the otolith (Campana and Gagné 1994). The most rapid and sensitive instrumentation for otolith analysis reported to date (Edmonds et al. 1992; Campana and Gagné 1994), and the instrument of choice for simultaneously quantifying the concentration of multiple elements and isotopes (Houk 1986; Date 1991), is inductively coupled plasma mass spectroscopy (ICPMS).

Analytical techniques that probe otolith composition in a focused beam are better suited to assaying the otolith nucleus (corresponding to the hatch site) than are whole-otolith assays, but generally suffer from poorer sensitivity. The electron microprobe is widely used for such assays and has had some success in differentiating among populations (Kalish 1990; Gunn et al. 1992), but is limited to the detection of only the six most abundant elements in the otolith (Kalish 1990). Probe techniques with slightly greater sensitivity, such as the proton microprobe, have also been used with success (Sie and Thresher 1992). However, sensitivity constraints have, until now, denied probe-based assays the same level of stock differentiation capabilities that has been available to solution-based assays. The recent development of a laser-based version of ICPMS may potentially overcome these restrictions, since it combines the beam capabilities of a high-energy laser with the analytical capabilities of ICPMS (Denoyer et al. 1991).

Conceptually, laser ablation – inductively coupled plasma mass spectroscopy (LA-ICPMS) can be envisioned as a traditional, solution-based ICPMS where sample is provided not as a solution, but as vaporized material ablated by a high-energy laser. The pulsed Nd:YAG laser (1064 nM) is focused onto a small area (nominal diameter 30–100 μm) of the sample solid, completely vaporizing the exposed surface to a depth of a few micrometres. The vaporized material is then swept by a flow of argon gas into the plasma of an ICPMS, where its relative elemental and isotopic composition can be accurately determined to at least the parts per million level. Both the sample and the ablation event can be viewed remotely in real time with 40× optics on a video camera and monitor. Electronic stepper motors attached to the sample stage allow for accurate and repeatable positioning of the sample. No sample preparation is required (once the surface of interest has been exposed), and the analysis can be done under atmospheric pressure.

The objective of this study was to provide a preliminary test of the stock differentiation capabilities of elemental fingerprints of the otolith nucleus determined with LA-ICPMS. Samples of Atlantic cod (*Gadus morhua*), collected from spawning grounds throughout the northwest Atlantic, served as the test species. Considerable attention was given to analytical sources of error and variability which could influence either the accuracy or the precision of the assays.

Materials and Methods

Otolith Collection

The sampling program was designed to catch fish of known stock through capture on the spawning ground in spawning condition. It is generally assumed that cod stock mixing is minimal at the time of spawning and that seasonal feeding migrations begin long after spawning has been completed (Templeman 1962). While not all of the cod used in this study were in spawning condition, most were preparing to spawn or had just spawned. Accordingly, we believe that our samples are reasonable representations of a number of discrete spawning stocks. With the exception of the Fundyrip–Georges Bank stock pair, all of the stocks are assumed to have remained geographically separated at all life history stages.

Cod were either collected at sea aboard research vessels using otter trawl gear or sampled from commercial catches where the fishing location was known with certainty. All samples were restricted to fish in the size range of 45–85 cm fork length to restrict the analysis to sexually mature individuals and to reduce variability caused by size-related effects. Subsamples of 30 fish from each of five sites were randomly selected for otolith elemental assays (Table 1; Fig. 1). The collection site of the Iceland sample (not shown in Fig. 1) was several miles off the western coast of Iceland. All samples were collected in 1986. Sampling dates varied across several months because of the tendency for cold-water stocks to spawn later than warmwater stocks.

Immediately after capture, fork length, head length (from the tip of the snout to the posterior end of the preoperculum), sex, and state of sexual maturity were recorded. The head was then severed, labelled, and frozen for subsequent otolith removal in the laboratory. All three otolith pairs (sagittae, lapilli, and asterisci) were removed from each fish, cleansed of adhering tissue, and stored dry in glass

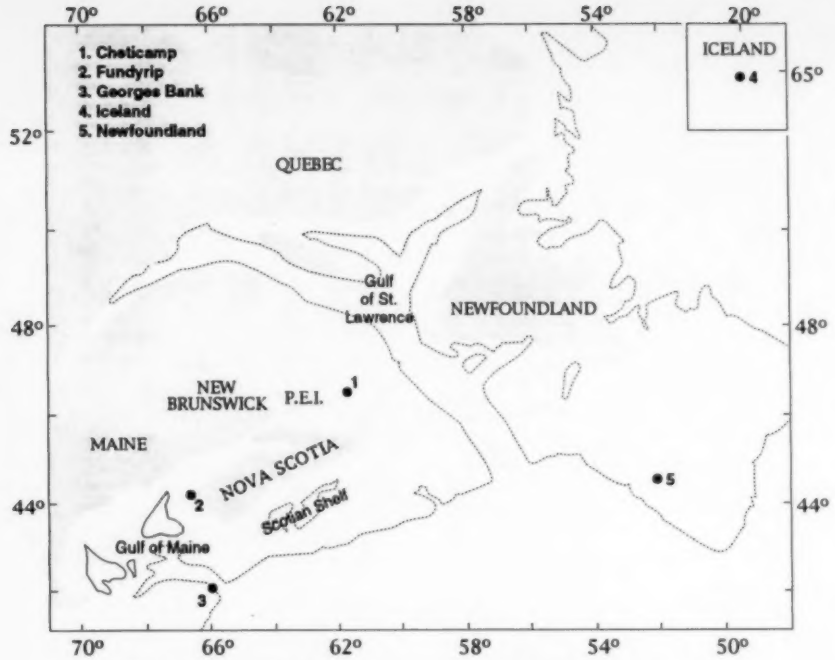

FIG. 1. Map of the study area, sampling sites, and 200-m contour.

vials until they could be examined further. Broken and crystalline otoliths were discarded (<1% of the sample).

Otolith Preparation

One lapillus per fish was prepared for solution-based elemental analysis with ICPMS, as has been reported elsewhere (Campana and Gagné 1994). The matching sagittae were embedded in polyester resin, sectioned transversely (Strong et al. 1985), and aged as per established procedures (Campana and Casselman 1993). One side of each sagittal thin section was subsequently polished with aluminum oxide lapping films as close as possible to the nucleus, using internal morphology as an index of proximity to the nuclear plane. Due to their opacity and the manner in which the otoliths had originally been sectioned, we often found it difficult to accurately locate the nucleus, particularly for the sample from Newfoundland. Accordingly, we abandoned efforts to polish to within 30 μm of the nucleus and instead targeted a plane 0–250 μm above that of the nucleus. Given otolith growth rates typical of cod off southwest Nova Scotia (Campana 1989), our final plane corresponded to a fish size of 3–22 mm and a fish age of 0–3 mo.

To remove surface contamination, sagittal sections were sonified for 5 min in Super-Q water (water that has been deionized, further purified through reverse osmosis, and then Millipore-filtered). After air drying, the sections were stored in paper envelopes and subsequently handled only with nonmetallic objects. More rigorous surface decontamination occurred later through laser-based preablation of the sample surface.

Laser Ablation

Prior to assay, otolith sections were preablated with a 6 × 6 rastered pattern of 60-μm craters in the nuclear region so as to remove any remaining surface contamination. Preablation was conducted while the sample cell was disconnected from the ICPMS to minimize unnecessary buildup of otolith material inside the ICPMS. Preablation craters were overlapped to minimize the possibility of fractionation at the crater edge (Krajnovich et al. 1993). Assays were subsequently conducted in the centre of the preablated region using 20 Q-switched energy pulses (<20 mJ) at 1-s intervals and a nominal beam diameter of 30 μm. Crater depth as measured with an atomic force microscope was about 30 μm, but the energy of the laser often shattered the surrounding region. Argon carrier flow rate was 1.5 $L \cdot min^{-1}$. Isotope quantification was based on 20 channels per amu, 7-s peak integration, and a dwell time of 320 μs. Argon gas blanks were run immediately prior to each assay and subsequently subtracted from the corresponding sample values. In principle, all blanks should incorporate a laser pulse due to the possibility of shock waves dislodging minute particles in the tubing leading to the ICPMS. However, preliminary tests using LA-ICPMS to ablate Teflon caps indicated that the latter contained significant levels of Rb, Zn, and Ba but were otherwise similar to Ar blanks.

The high Ca content of the otolith material caused significant buildup on the nebulizer tips, sampler, and skimmer cones, as well as on the inside of the quadrupole. As a result, instrument drift was unavoidable, resulting in detection limits that were elevated somewhat over normal levels. The ICPMS was recalibrated periodically, but drift was evident for most elements, particularly after recalibration. Drift was most evident when relative elemental concentration was plotted as a function of order in the analysis sequence. To avoid the possibility of confounding instrument drift effects with stock differences, samples were assayed in a systematic order, e.g., sample from Stock 1, sample from Stock 2,

TABLE 2. Elements and isotopes detectable with LA-ICPMS in cod otolith nuclei. All measurements were significantly above background levels. Isotopes accepted for use in the analysis are marked with an asterisk.

Element/ isotope	Natural abundance, %	Isotope counts relative to background counts	Analytical reliability
B-10*	19.78	1.30	—
B-11	80.22	1.35	Spillover from C-12
Mg-24*	78.70	12.36	—
Mg-25*	10.13	9.89	—
Al-27	100.00	~1	Interference from Al stage
Ca-46*	0.004	7.18	—
Ca-48*	0.187	86.43	—
Fe-57*	2.19	1.18	Interference from Ar-OH?
Zn-66*	27.81	3.31	—
Br-81	49.46	~1.5	High background
Rb-85*	72.15	1.80	—
Sr-86*	9.86	284	—
Sr-87*	7.02	>1000	—
Sr-88*	82.56	>1000	—
Ag-107	51.82	~10	Probably Ar–Al (no Ag-109)
Sn-118*	24.03	40.05	—
Ba-137*	11.32	>1000	—
Ba-138*	71.66	>1000	—

sample from Stock 3, etc. The effect of instrument drift was later effectively removed as part of the statistical analysis.

Solution-based ICPMS can be calibrated by spiking bulk sample solutions with known concentrations of elemental standards. This approach is effective in spite of the potential influence of the sample matrix on detection efficiency and molecular ion interference. However, spiking of solid samples is not possible in the same manner. Many electron microprobe analyses use Ca as an internal standard against which other elemental concentrations are referenced (Kalish 1989; Gunn et al. 1992), despite the fact that the ratios are not equivalent to the ratios of their concentrations (due to interchannel differences in detection efficiency). A similar approach (using Ca-48 as the internal standard) could also have been used in this study, in light of the low interotolith Ca concentration variability found in cod otoliths (Campana and Gagné 1994). This type of standardization would not provide absolute elemental concentrations, but would remove sample to sample variability in laser energy and the weight of ablated material. However, we elected instead to adjust for the quantity of ablated Ca-48 through its incorporation into the statistical model as a covariate. As it turned out, statistical analyses of the data using both approaches revealed no appreciable differences between the two.

Analytical reproducibility of the laser ablation system was tested using replicate assays of known-composition glass (NBS 615). Ten spots were sequentially laser-sampled on each of 4 d in an ANOVA design. The isotopes assayed were the same as those assayed in the otoliths.

Statistical Analyses

All isotopic data were first examined for normality and ln–transformed where appropriate. The statistical analysis was designed to adjust for both instrument drift between ICPMS adjustments and variations in the amount of ablated material (as indexed by Ca-48). The resulting MANOVA included 14 isotopes as dependent variables, sequence block and sample site as factors, and Ca-48 as a covariate. Interaction terms were nonsignificant and therefore dropped from the model. Factor analysis, discriminant analysis and univariate ANOVA's were used to determine the isotopes most influential in the analysis. Classification success to each of the sample sites was determined through jackknifed discriminant analysis.

Results

Analytical Sensitivity and Precision

Isotopes were selected for subsequent assay after completion of a full elemental scan of both otolith material and an Ar blank. All isotopic measurements that were significantly above background levels ($p < 0.01$) were then assessed for isobaric interferences (particularly from Ar, Ca, and N molecular ions) and spillover from adjacent abundant isotopes. Only those isotopes that were free from interferences were used in subsequent analyses. A total of 14 isotopes, representing nine elements, could be measured with confidence (Table 2).

Incorporation of Ca-48 as a covariate in the statistical analysis is equivalent to using it as an internal standard. Yet, standardization of elemental concentrations against Ca concentration is only justifiable if the latter is a measure of ablated mass and is relatively invariant across otoliths. Ca-48 was highly significant ($p < 0.001$) as a covariate in the MANOVA (Table 3). In addition, univariate regressions of unstandardized isotope concentrations against Ca-48 were significant ($p < 0.05$) in all cases (except B-10), indicating that isotope concentrations increased with Ca-48 concentration. These results suggest that Ca-48 concentration is indeed a measure of ablated mass and that standardization to Ca-48 should remove sample-to-sample variation in laser energy and ablated mass. To confirm this, nested ANOVA's of each isotope, using paired otoliths (left and right from each fish), were used to partition the variance between fish effects and individual otolith and/or sample processing effects. Comparison of nested ANOVA results for each isotope

TABLE 3. Results of the MANOVA testing for effects of sequence in the analysis, ablated mass, and stock on the elemental fingerprint of the otolith nucleus.

Source of variation	Pillais value	Hypothesis df	Error df	*F*–ratio	Significance
Sequence block	1.48	52	484	5.48	0.000
Sample site	0.66	52	484	1.84	0.001
Ca-48 (covariate)	0.55	13	118	11.1	0.000

TABLE 4. Concentrations of isotopes of interest in a known-composition glass material (NBS 615) as measured with LA-ICPMS on four different days (n = 10 per day). Nominal concentrations were not available for some isotopes.

Isotope	Nominal concentration, ppm	Mean count	Within-day CV, %	Across-day CV, %
B-10	0.26	676	30	67
Mg-24	—	8 861	11	20
Mg-25	—	1 327	40	46
Ca-46	3	2 735	18	35
Ca-48	160	83 051	6	15
Fe-57	0.29	−1 392	45	144
Zn-66	—	508	40	86
Rb-85	0.62	600	20	40
Sr-86	4.52	2 254	8	19
Sr-87	3.22	1 823	6	19
Sr-88	37.81	19 228	7	21
Sn-118	—	1 344	40	126
Ba-137	—	218	13	21
Ba-138	—	1 400	8	23

TABLE 5. Nested ANOVA's of isotope concentrations in 62 paired otoliths from 31 cod. The percentage of the variation explained by the fish is a reflection of the isotope's reliability as a component of the fish's elemental fingerprint.

	Sum of squares		df		Variation %	
Isotope	Fish	Otolith	Fish	Otolith	Fish	Otolith
B-10	6.001	1.187	30	31	67.9	32.1
Mg-24	4.715	1.966	30	31	42.5	57.5
Mg-25	8.636	4.961	30	31	28.5	71.5
Ca-46	3.897	2.273	30	31	27.9	72.1
Fe-57	2.103	0.356	30	31	71.9	28.1
Zn-66	3.323	2.422	30	31	17.3	82.7
Rb-85	1.032	0.541	30	31	32.6	67.4
Sr-86	2.076	0.425	30	31	66.9	33.1
Sr-87	3.152	0.487	30	31	74.0	26.0
Sr-88	6.692	1.679	30	31	60.9	39.1
Sn-118	5.380	0.725	30	31	76.9	23.1
Ba-137	4.957	0.886	30	31	70.5	29.5
Ba-138	2.023	0.290	30	31	75.6	24.4

before and after standardization to Ca-48 indicated that the standardized contribution due to fish effects exceeded that of the unstandardized contribution for all isotopes except two (Ca-46 and Rb-85), confirming the value of standardizing isotope concentrations to Ca-48. However, the improvement was very modest, generally less than 10%.

Position in the analysis sequence had a very large effect on apparent isotopic concentrations. Plots of concentration against sequence number for each isotope revealed large, abrupt shifts in concentration associated with changes in ICPMS operating conditions. All were associated with plasma shutdown (e.g., at lunch and at the end of the day), but were not due to complete shutdown of the ICPMS. Since the Ar blanks showed similar shifts in concentration, the discontinuities were associated with ICPMS operation and not the laser. Shifts in isotope concentrations due to these effects were not parallel across isotopes, i.e., one isotope might increase and another decrease at plasma shutdown, while a third might stay constant. However, the differences tended to be both large and significant, whether analyzed individually or in the MANOVA (Table 3). It is important to note that the magnitude of the isotope concentration shifts associated with the ICPMS far exceeded the sample-to-sample or stock-to-stock variability. Failure to systemically mix samples across the analysis sequence, or to include a sequence factor in the statistical analysis, would have introduced huge variances into the data and could have introduced significant but artifactual differences among samples.

Analytical reproducibility of the laser ablation system was further tested using replicate assays (n = 10) of known-composition glass (NBS 615) on each of 4 d. Within any given day, the coefficient of variation (CV) ranged from less than 10% (for elements such as Ca, Sr, and Ba) to more than 100% (for elements such as Fe and Sn), with an overall mean of about 20% (Table 4). All isotopes showed large and highly significant ($p < 0.01$) differences across days. CV's across days were generally 2–3 times larger than those within days for any given isotope. Standardization to Ca-48 did not eliminate the significant day effect, although it did reduce across-day CV for two of the elements (Sr and Ba). These results confirm the influence of ICPMS operation (independent of laser operation) on analytical results, validate the necessity of statistically removing sequence effects, and document the necessity of systematic mixing of the

TABLE 6. Mean otolith isotopic concentrations, adjusted for Ca-48, by sample site. Variables that were ln-transformed are marked (ln). Sample size was $n = 28$ for all sites except Cheticamp, where $n = 29$. Isotopes that differed significantly ($p < 0.05$) across sites are marked with an asterisk. Where present, significant pairwise differences with another site (represented by its first letter) are indicated in parentheses.

	Sample site				
Element/isotope	Cheticamp	Fundyrip	Georges Bank	Iceland	Newfoundland
B-10 ($\times 10^4$)*	1.8	1.7 (N)	2.4	2.6	4.1 (F)
Mg-24 ($\times 10^2$)*	3.44 (G, I)	3.78	3.97 (C)	4.08 (C)	3.78
Mg-25 ($\times 10^3$)	4.87	5.27	5.09	5.49	4.95
Ca-46 ($\times 10^2$)	1.44	1.44	1.44	1.45	1.44
Fe-57 (ln)	−5.92	−5.92	−6.01	−6.09	−5.98
Zn-66 (ln)	−7.61	−7.70	−7.86	−7.49	−7.84
Rb-85 ($\times 10^5$)	5.2	3.3	4.2	4.6	5.7
Sr-86 (ln)*	−1.67 (I)	−1.72 (I)	−1.76 (I)	−1.56 (C, F, G, N)	−1.75 (I)
Sr-87 (ln)*	−1.67	−1.77 (I)	−1.74 (I)	−1.59 (F, G, N)	−1.78 (I)
Sr-88 (ln)*	0.43	0.40 (I)	0.39 (I)	0.50 (F, G, N)	0.39 (I)
Sn-118 ($\times 10^4$)	8.8	8.1	8.3	7.9	7.4
Ba-137 (ln)*	−8.13	−7.83 (I, N)	−8.21	−8.49 (F)	−8.26 (F)
Ba-138 (ln)*	−6.13	−5.83 (G, I, N)	−6.22 (F)	−6.45 (F)	−6.22 (F)

order of sample analysis. However, because of compositional differences in the sample matrix, the precision of the glass assays is not necessarily representative of that of the otolith assays.

Comparison of isotope concentrations between left and right otoliths from the same fish indicated that the elemental fingerprints were at least partially representative of the fish, and not just the laser sampling site or the otolith. Nested ANOVA's of the paired otolith data indicated that 17–77% of the partitioned variance was due to fish effects (Table 5). On the basis of these results, it is reasonable to conclude that concentrations of elements such as B, Fe, Sr, Sn, and Ba can be considered to be elemental signatures of the fish, rather than just the individual otolith. However, elements such as Ca, Zn, and Rb provided relatively weak signatures.

Reproducibility of the isotopic signatures was also assessed through comparison of multiple isotopes within any given element. Correlations of within-element isotopic concentrations were all highly significant ($p < 0.0001$) (Mg-24/Mg-25, $r = 0.78$; Sr-86/Sr-87, $r = 0.84$; Sr-86/Sr-88, $r = 0.87$; Sr-87/Sr-88, $r = 0.70$; Ba-137/Ba-138, $r = 0.96$), indicating that isotopic values were indeed reflecting true elemental abundances. A poor correlation would have suggested interference in the quantification of one or more of the isotopes. Indeed, poor correlation between the two isotopes of B (B-10 and B-11) formed part of the basis for rejecting the use of the latter.

Elemental Fingerprint Differences among Sites

The elemental fingerprints of the otolith nuclei differed significantly across sites (MANOVA, $p < 0.001$) (Table 3). Univariate F-tests indicated that the concentrations of B-10, Mg-24, Sr-86, Sr-87, Ba-137, and Ba-138 all differed significantly across sample sites ($p < 0.05$). A posteriori contrasts indicated that the Fundyrip site often differed significantly from the Iceland and Newfoundland sites and that the Iceland site often differed from Fundyrip, Georges Bank, and Newfoundland (Table 6). Despite the statistical significance, many of the intersite differences were relatively small, typically less than 50% of the mean (Table 6). None of the within-element isotope ratios differed significantly among sites.

Neither age, sex, nor fish length accounted for the intersite differences in isotope concentration. Three-way ANOVA's of each individual isotope concentration, using sequence block, site, and sex as factors, demonstrated that sex was not significant ($p > 0.10$). Since age compositions did not necessarily overlap between sites, it was not possible to carry out similar analyses for age effects. However, within-site analyses of individual isotopes demonstrated that age had no significant relationship with isotopic concentration (ANOVA, $p > 0.10$). Similarly, fish length was not a significant covariate in the analysis of intersite differences (MANOVA, $p > 0.05$).

Stepwise discriminant analyses of the elemental concentration data confirmed the significant discrimination among sample sites ($p < 0.05$). The stepwise variable selection included only three of the 18 possible isotopes and isotope ratios: Ba-138, Sr-86, and Mg-24. Pairwise differences were similar to those associated with the MANOVA contrasts, where Iceland differed significantly from all of the other sites, and Fundyrip differed from Cheticamp and Georges Bank at the $p = 0.06$ level. However, classification accuracy was relatively low, with a mean jackknifed accuracy estimate of 30%. There was no apparent tendency for misclassified samples to be classified to the nearest alternative sample site.

Discussion

Elemental fingerprinting of otolith nuclei appears to have potential for differentiating among cod populations. Cod spawn on dozens of offshore banks and in many coastal regions throughout the northwest Atlantic, with each spawning aggregation assumed to represent a distinct population (Templeman 1962). However, cod are highly migratory and many populations intermix at times other than spawning (McKenzie 1956; Templeman 1962; Wise 1963), thus confounding past attempts to distinguish among stocks. Genetic differentiation has, in general, proven unsuccessful in differentiating among multiple cod stocks (Cross and Payne

1978; Mork et al. 1985; Smith et al. 1989; but see Carr and Marshall 1991). Tagging (Wise 1963), morphometrics (Bowen 1987), meristics (Lear and Wells 1984), parasite loads (Scott and Martin 1957), and ichthyoplankton surveys (O'Boyle et al. 1984) have all been used to confirm the presence of multiple cod stocks, but none has provided a reliable measure of stock identity. The results of this study provide encouraging indications that elemental fingerprints of the otolith nucleus may prove useful for differentiating among cod stocks. While technical limitations to our ability to more completely retrieve the information locked in the otolith nucleus remain, these limitations appear to be tractable.

Various workers have reported sample-specific differences in elemental concentration in tissues other than otoliths, but the implications of their findings are somewhat unclear. Analyses of bone (Behrens Yamada et al. 1987; Hamilton and Haines 1989; Miller et al. 1992), scales (Johnson 1989), and various soft tissues (Calaprice 1971; Hellou et al. 1992) must reflect composition during growth, but also incorporate metabolic reworking after initial deposition as well as tissue depuration. As a result, the temporal stability of the elemental concentrations in nonotolith tissues is questionable. In contrast, the acellular otolith is metabolically static (Campana and Neilson 1985) and is not exposed to outside water, making it an ideal storage site for trace elements after incorporation.

Otolith elemental fingerprinting has proven successful in detecting intersite differences in a variety of fish species. Edmonds et al. (1989, 1991, 1992) reported site-specific differentiation of several Australian fishes. Site-specific elemental concentrations were also reported by Mulligan et al. (1987), Grady et al. (1989), Gunn et al. (1992), Secor (1992), Sie and Thresher (1992), and Campana and Gagné (1994), although the validity of the Mulligan et al. (1987) study now appears questionable (Kalish 1990; Gunn et al. 1992). Kalish (1990) used otolith elemental fingerprints to distinguish between anadromous and nonanadromous salmonids from the same site. In all of the above studies, it was not clear if the elemental fingerprints were environmentally driven or incorporated a genetic component. However, the validity of using stock- and site-specific fingerprints does not rest upon the mechanism underlying their formation.

Comparison of the current cod stock discrimination results with those of a sister study based on ICPMS analysis of whole dissolved lapilli (Campana and Gagné 1994) provided results that were, at least initially, counterintuitive. Many of the same isotopes differed significantly among sites in both studies. However, the elemental fingerprints of whole otoliths were far more powerful in distinguishing among the cod stocks than were laser-based assays of the same fish using the otolith nucleus. Technical reasons for the discrepancy included the greater sensitivity and enhanced precision of solution-based ICPMS compared with LA-ICPMS (van Heuzen 1991; Hall 1992; Jackson et al. 1993). In addition, the sister study failed to randomize the sample assay sequence (as was done here), which may have contributed artifactually to apparent stock discrimination success. Finally, our inability to precisely section the otolith through the nucleus undoubtedly interfered with our LA-ICPMS assay results, if only because the resulting elemental fingerprint came to represent variously the fingerprint of the egg, larva, or early juvenile, rather than just the egg. There are also theoretical explanations for the different results, not the least of which is a different underlying hypothesis. Stock differentiation based on whole-otolith fingerprints takes advantage of differences in home range (with associated differences in water composition), as well as differences in spawning site. Under Ihssen et al.'s (1981) definition of a stock as an "intra-specific group of randomly mating individuals with temporal or spatial integrity", the progeny of a single spawning aggregation could grow up in a range of different environments, with relatively little intermixing after hatch. Such stocks would likely be characterized by different whole-otolith elemental fingerprints, just as they would demonstrate other morphological differences, such as growth rate. Conversely, application of elemental fingerprinting to otolith nuclei tests only for differences in or around the hatch site; the presence or absence of differences in posthatch environment plays no part. Thus, the fingerprint of the nucleus is more closely akin to a test for genetic differences than is the whole-otolith fingerprint, despite the fact that subsequent spatial segregation would serve only to amplify the differences in otolith elemental composition.

Despite the statistically significant stock discrimination achieved with LA-ICPMS in this study, classification success was only moderate at best. This was surprising, given that all but one pair of the tested stocks (Fundyrip and Georges Bank) are believed to be completely discrete at all life history stages. More importantly, misclassified individuals were not necessarily identified as being from adjacent, or even environmentally similar, stocks. Explanations for the above are not obvious, although several levels of variability undoubtedly contributed to each reading of elemental composition:

(1) Instrumental error: the CV's documented in our experiments with the known-composition glass highlight the variability that is possible in this type of study, although matrix effects make comparisons of glass and otolith material difficult (Perkins et al. 1991).

(2) Preparation and crystal-level error: the statistical power of this study was lower than expected because of the variability introduced during the sectioning. However, the nested ANOVA's indicated that most isotopes were relatively stable components of the elemental fingerprint, as demonstrated by their parallelism between the left and right otoliths of any given fish. Therefore, the fingerprints were real, and not just instrumental artifacts.

(3) Variability among fish collected at the same site: year-to-year differences in seawater composition would be expected to induce different elemental fingerprints in fish of different age, and thus reduce our ability to differentiate among stocks. Although we did not detect any significant age effects in the analysis, that does not mean that they were not there. In addition, the incorporation of trace elements into calcium carbonate is, at least in part, a function of crystallization rate (Lowenstam and Weiner 1989) and growth rate, implying that differences in physiological condition between fish of a given stock contributed to increased variance. This hypothesis requires testing. Also possible is a violated assumption of site fidelity, wherein adults were assumed to have returned to their hatch site to spawn. This assumption is difficult to test in nonanadromous species, but is almost certainly invalid to a certain extent (Sinclair 1988). Violations of the site fidelity assumption would result in greatly reduced stock discrimination ability, since the adult fish caught at one site would actually represent a mixture

from different sites. Note that this assumption is far more critical to this type of study than to whole-otolith assays, given that fish eggs and larvae are susceptible to large-scale transport and diffusion processes.

(4) Variability among sites: this was the level of variability that was of greatest interest to us. Among-site differences are dependent on differences in the physical characteristics and composition of seawater, which may or may not come with increased geographic separation. For instance, water from the Gulf of St. Lawrence eventually feeds into the Bay of Fundy (our Fundyrip sample), linking the two widely separated sites after a lag time of several months (Smith 1983). Similarly, water from the Gulf Stream feeds both Georges Bank and Iceland, and thus may reduce the expected difference in seawater composition between the two areas. Water temperature also varies among sites, but does not necessarily decline with increasing latitude. However, there is no question that all of the sites differed in physical and chemical composition to some degree, and our results suggest that those differences were at least partially reflected in the otolith. Any physiological or genetic differences between stocks that influenced otolith elemental uptake may also have contributed to among-site differences in the elemental fingerprint.

Laser ablation ICPMS offers numerous advantages, and some disadvantages, over alternative instrumentation available for characterizing an elemental fingerprint of the otolith nucleus. The primary advantages are those of minimal sample preparation and the simultaneous determination of both isotopic and elemental concentrations, with a sensitivity that is unmatched by other probe techniques. While the sensitivity of solution-based ICPMS (<0.03 ppb, Houk 1986) greatly exceeds that possible with LA-ICPMS, the detection limits of <300 ppb observed in this study are conservative compared with those reported elsewhere for LA-ICPMS (Denoyer et al. 1991; van Heuzen 1991; van Heuzen and Morsink 1991; Hall 1992; Pearce et al. 1992) and several orders of magnitude better than the limits of about 3000 and 100 ppm possible with energy-dispersive and wavelength-dispersive electron microprobes, respectively (Denoyer et al. 1991; Gunn et al. 1992). The detection limit of the proton microprobe is of intermediate value, around 1 ppm (Ishikawa et al. 1987). However, LA-ICPMS does not suffer from the charging problems that can confound either electron or proton microprobe assays, nor does it require a smoothly polished sample surface as do the other techniques. Disadvantages of LA-ICPMS include the inability to accurately measure the abundance of some of the lighter, relatively abundant elements, interference of polyatomic ions with certain isotopes, and the potential for instrument drift due to Ca buildup during sequential assays. The latter constraint can be overcome by insuring that the order of sample assays is statistically blocked, crossed, and balanced with respect to sample site, as was done in this study. However "memory effects", due to transient particle deposition in the transfer tubes (van Heuzen 1991), are more problematic, and undoubtedly contribute to CV's that are somewhat greater than those observed with either conventional ICPMS (Jackson et al. 1993) or an electron microprobe. Spatially, the 30-μm spot size of the laser cannot compete with the 3-μm spot size of an electron microprobe (Gunn et al. 1992). As is the case with other probe techniques, it is difficult to provide quantitative estimates of elemental concentration with LA-ICPMS, although some workers have reported limited success through use of standard-spiked pressed tablets made from sample material (Perkins et al. 1991; van Heuzen and Morsink 1991; Pearce et al. 1992).

Aside from continued effort to improve the accuracy and precision of LA-ICPMS, we can identify several issues in elemental fingerprinting requiring additional research. Ontogenetic effects on otolith composition are not yet clear, nor has the source of the trace elements (water versus diet) been identified. The role of physiological regulation in controlling the uptake of trace elements into the otolith is poorly understood, even though Kalish (1989) has helped define the factors influencing the uptake of major and minor elements. Experiments to test the response of otolith trace element composition to variations in water composition are clearly required. By corollary, confirmation that otolith elemental composition reflects seawater composition at the hatch site is lacking. Although large-scale spatial variations in seawater composition exist (Johnson et al. 1992), we were not able to find any reports of large-scale spatial variation in the isotopes of interest in the northwest Atlantic. Increased interaction between chemical oceanographers and those using otolith elemental fingerprints would undoubtedly be of value.

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Sediment–Water Distribution Coefficients and Speciation of Cadmium in a Canadian Shield Lake[1]

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Wagemann, R., M.J. Capel, R. Hesslein, and M. Stephenson. 1994. Sediment–water distribution coefficients and speciation of cadmium in a Canadian Shield lake. Can. J. Fish. Aquat. Sci. 51: 1951–1958.

Sediment–water distribution coefficients for Cd were strongly pH dependent at pH > 6.5 for three surficial bottom sediments and suspended particles from a Canadian Shield lake (Lake 382). A single exponential equation in terms of pH fitted the distribution coefficients of all sediments. In the range $6.5 \leq pH \leq 8$ the distribution coefficients were 109×10^{-3} to 1579×10^{-3} L/mg for suspended particulate matter and 0.6×10^{-3} to 129×10^{-3} L/mg for the bottom sediments. Speciation calculations performed using the computer program MACS80 and measured Cd fractions associated with suspended particles agreed well down to 7 m. In deeper water the measured fraction exceeded the calculated fraction, hypothetically because of CdS formation, differing sediment compositions, or nonequilibrium conditions. In September, H_2S was 100–300 μg/L in the lower hypolimnion. Even at only 5 μg dissolved sulfide/L, some of the added Cd would precipitate as CdS at pH > 6, and the dissolved Cd, under anoxic conditions, would be largely $Cd(HS)^+$ and $Cd(HS)_2$. Under aerobic conditions at pH ≥ 7.5, approximately 85% of the Cd was on suspended particles and complexed with dissolved organic matter. The solids $Cd(OH)_2$, $CdCO_3$, and $Cd_3(PO_4)_2$ played no role in the control of dissolved Cd.

À un pH > 6,5, les coefficients de distribution sédiment–eau du Cd de trois échantillons de la surface du fond et des particules en suspension d'un lac du Bouclier canadien (lac 382) dépendaient fortement du pH. Pour décrire le comportement du pH, une seule équation exponentielle décrivait les coefficients de distribution de tous les sédiments. Dans la plage $6,5 \leq pH \leq 8$, les coefficients de distribution étaient de 109×10^{-3} à $1\,579 \times 10^{-3}$ L/mg pour les matières particulaires en suspension et de $0,6 \times 10^{-3}$ à 129×10^{-3} L/mg pour les sédiments de fond. Les calculs de spéciation effectués à l'aide du programme informatique MACS80 et les fractions mesurées de Cd associées aux particules en suspension présentaient un bon accord jusqu'à 7 m. Dans les eaux plus profondes, la fraction mesurée dépassait la fraction calculée, théoriquement à cause de la formation de CdS, de compositions de sédiments différentes et de conditions non à l'équilibre. En septembre, la teneur en H_2S était de 100–300 μg/L dans l'hypolimnion inférieur. Même à une concentration de sulfure dissous de seulement 5 μg/L, une certaine partie du Cd ajouté précipité à l'état de CdS à un pH > 6, et le Cd dissous, dans des conditions anoxiques, se trouvait surtout à l'état de $Cd(HS)^+$ et de $Cd(HS)_2$. Dans des conditions aérobies à pH ≥ 7,5, environ 85 % du Cd se trouvait sur les particules en suspension et complexé avec la matière organique dissoute. Les solides $Cd(OH)_2$, $CdCO_3$ et $Cd_3(PO_4)_2$ ne jouaient aucun rôle dans la régulation du Cd dissous.

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In natural aquatic systems the simple total metal concentration in water is seldom sufficient of itself to elucidate effects on organisms, or explain biological availability of metals. The chemical speciation of metals in solution and the partitioning of metals between the aqueous and solid phases influence the biological availability and toxicity to organisms, as well as the geochemical fate of metals. The forms of occurrence of metals need to be known in order to understand the processes taking place in natural systems. The chemical processes that determine the concentration and chemical form of metal species are those of complex formation, solution–precipitation, and adsorption. An important factor determining the residence time in the water column of chemical species is adsorption at the particle–water interface. The more reactive the species, the more it will be bound to particles, the more rapidly it will be removed, and the shorter will be its residence time in the water column (Hesslein et al. 1980; Hesslein 1987; Honeyman and Santschi 1988) and therefore its availability to nonbenthic organisms.

In practice it is very difficult to measure the concentration of individual molecular and ionic species of metals in natural water. The ion-specific electrode can in principle measure "free" metal ions, but in practice it is insufficiently sensitive for Cd and all but a few metals to give reliable measurements at neutral or basic pH at metal concentrations encountered in freshwaters. Polarographic techniques

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TABLE 1. Average composition of water, suspended particles, and bottom sediments of Lake 382 from three different locations within the lake.

	Na, mg/g	K, mg/g	Mg, mg/g	Ca, mg/g	Fe, mg/g	Mn, mg/g	Si, wt %	Cl	SO_4	TDIC[a]	TOC,[b] mg C/g	SPM,[c] mg/L	pH
Bottom sediments O	15.0	12.7	4.59	12.0	23.3	0.46	22.7	—[d]	—	—	129	—	—
Bottom sediments Q	10.6	12.6	4.44	11.0	23.6	0.42	24.1	—	—	—	110	—	—
Bottom sediments B	4.42	6.18	3.37	6.10	19.3	0.41	20.8	—	—	—	158	—	—
Suspended particles[f]													
Hypolimnion	—	—	1.11	8.83	87.7	19.4	—	—[e]	—	—	—	—	—
Epilimnion	—	—	1.06	7.69	4.56	0.44	—	—[e]	—	—	—	—	—
Water, mg/L[g]	1	0.5	0.7	2.5	0–1	0–0.3	—	0.3	3	0.1–3.6	500–900[b]	1–5	5.3–7.3

[a]TDIC = total dissolved inorganic carbon, mg C/L. TOC = total organic carbon.
[b]TDOC = total dissolved organic carbon, μM.
[c]Concentration of suspended particles in the water column.
[d]Analyses not performed.
[e]Analyses not performed due to insufficient sample.
[f]Elemental composition of suspended particles; integrated sample from 1–7 m for the epilimnion, 7–11.5 m for the hypolimnion.
[g]Lake 382 water, ionic strength (I) = 0.0036 by conductivity.

(e.g., anodic stripping voltametry) are very sensitive and have been used successfully to differentiate groups of labile and nonlabile species (Florence and Batley 1977), but are unsuited operationally to determine equilibrium concentrations of individual metal species because of their requirement for buffering of the sample prior to measurement which may cause a shift in the equilibrium distribution of the metal species. For example, in the more acidic environment of a buffer (acetate buffer is commonly used), some species dissociate and the "free" metal concentration increases. The computational approach avoids this problem, but does suffer from a scarcity of reliable constants for some metal species, e.g., metals adsorbed on suspended matter as a function of pH and metals associated with organic matter. The adsorption process has therefore often been neglected in computer modelling of chemical equilibria of metals in natural waters. The MACS80 chemical speciation computer program (Wagemann et al. 1990), which does take into account the interaction of Cd with particulate matter and humic acid (and over 300 other species), was used in this investigation.

Much information has been compiled on the adsorption of metals on various simple substrates (Schell et al. 1981; Rai and Zachara 1984), of radionuclides in marine environments (Li et al. 1984; Nyffeler et al. 1984; IAEA 1985), and of metals in rivers (Gardiner 1974; Ramamoorthy and Rust 1978; Tang and Xue 1983; Zhang et al. 1990), but very few Cd distribution coefficients are available for lacustrine environments (Sigg et al. 1982; Dunn and Tissue 1987). Similarly, the explicit influences of solution pH and organic matter on the distribution coefficients appear not to be available for lacustrine systems.

The purpose of this investigation was to determine the steady-state sediment–water distribution coefficients for Cd in an oligotrophic Shield lake (Lake 382) as a function of pH using water and sediments from that lake and to compare the measured fraction of Cd adsorbed on suspended particles with the calculated fraction using the MACS80 chemical speciation program based on the Cd concentrations from additions of Cd to the lake, the normal major ions and constituents in the lake, and solubilities of the most insoluble solids involving Cd as a function of pH. Additions of Cd to the whole lake (up to 100 ng/L in the water column) began in the spring of 1987, in connection with other experiments (Malley et al. 1989), providing an opportunity for comparing calculated results with actual measurements, and in August 1987, water samples were taken from Lake 382 for the purpose of this investigation. The uptake of Cd by biota in lakes in this area had been studied (Marshall and Mellinger 1980; Marshall et al. 1981). The dynamics and effects of metals can vary considerably over time and space in stratified lakes concomitantly with a variation in pH. Surficial bottom sediments from different locations in the lake were therefore taken (prior to the addition of Cd to the whole lake) in order to see the variation in the distribution coefficient with sampling location within the same lake.

Methods

Lake 382 is a small (37 ha), oligotrophic, dimictic, soft-water Canadian Shield lake in the Experimental Lakes Area of northwestern Ontario at 49°42′N, 94°41′W. It is a headwater lake with two intermittent inflow creeks from a bog area and an outflow. It is situated in a wooded drainage area of predominantly spruce and jack pine.

Steady-state sediment–water distribution coefficients for Cd were determined at 6°C at different pH values using ^{109}Cd as a tracer in a laboratory setting open to the atmosphere. Three different bottom sediments (grab samples of determined composition from different locations approximately 400 m apart in Lake 382 (designated O, Q, and B from 3, 5, and 11 m, respectively), suspended sediment (an integrated sample from the epilimnion), and Lake 382 water were used without prior pretreatment (Table 1). The sediments and lake water, with ^{109}Cd added, were equilibrated for 1 wk. The radioactive Cd (Los Alamos National Laboratory, Material Management Division, P.O. Box 990, Los Alamos, NM 87545) was essentially carrier free. The contribution of Cd from this source to the natural background concentration of Cd in Lake 382 water (1–2 ng/L) was negligibly small.

The composition of this lake water was typically that of an oligotrophic Shield lake. The time required for Cd to be equilibrated between sediment and water, established in separate experiments, did not exceed 120 h for any of the

sediments. Sediments were kept frozen (together with lake water) until used. Suspensions of sediment in lake water were prepared and kept open to the atmosphere throughout the course of the experiments. In all experiments, final readings were not accepted until the pH had stabilized. The dependence of K_d on the concentration of particulate matter (Honeyman and Santschi 1988) was not investigated.

The Cd distribution coefficient (K_d) was calculated with the following formula:

$$(1) \quad K_d = \left(\frac{A_s \times \mathrm{gf}}{A_w} \right) \times \frac{V}{m}$$

where A_s = activity (counts per minute (cpm)) of Cd on suspended sediment, A_w = activity (cpm) of Cd in water, V = mass (grams) of water, m = mass (grams) of suspended sediment, and gf = geometry factor for the counter (0.8, filter to solution)

Since no specific ambient concentration of bottom sediments occurs in a lake, a sufficiently high concentration was used in laboratory experiments to minimize experimental errors. For each bottom sediment, nine 100-mL samples of filtered (0.45-μm pore size) lake water and sediment (100 mg/L) were prepared at 0.5 pH unit increments between pH 4.0 and 8.0, using either dilute HCl or $NaHCO_3$ for pH adjustment and initial maintenance. The final pH (after 1 wk) accepted for the experiment generally differed from the initially adjusted pH by 0.2–0.4 pH unit. ^{109}Cd (activity 3.7×10^{10} Bq/g Cd; 1.0 Ci/g Cd) was added to each bottle (approximately 3.7×10^3 Bq/bottle; 0.1 μCi/bottle) and maintained with gentle shaking at 6°C for 1 wk. Thereafter, each sample was filtered through a 0.45-μm, 4.5-cm-diameter Millipore® filter. The prewashed and preweighed filters with sediment were dried in a desiccator and weighed to obtain the weight of the sediment on each filter. The dried filters with the sediment were rolled up, placed in 10-mL plastic tubes, and counted (LKB-Wallac 1282 Compugamma) for 5 min or until 10 000 counts were reached. The filtrate (5 mL) was similarly counted.

For suspended sediment (approximately 1 mg/L), larger samples were used. Nine 1-L bottles of lake water with its indigenous suspended sediment were used and similarly adjusted for pH; ^{109}Cd was added (approximately 3.7×10^4 Bq/bottle; 1 μCi/bottle) and equilibrated. Two 400-mL and one 200-mL sample from each bottle were filtered and the dried filters and 5 mL of each filtrate were counted. Adsorption of ^{109}Cd on the filter was checked by filtering sediment–water samples through two filters simultaneously, one on top of the other. The number of counts on the second filter was insignificant compared with the counts on the top filter. The number of counts on the Nalgene bottles themselves (high-density polyethylene) used in these experiments was essentially zero. Average ± % counting errors in the cpm (over the pH range 4–8) for sediment–water were 1.4/2.5 (O), 1.4/2.3 (Q), 1.0/1.0 (B), and 0.9/1.0 (suspended matter). Although the water used with each sediment was the same (Lake 382), equilibration with different sediments resulted in somewhat different counting errors for water. The given errors in the distribution coefficients represent the most probable error, i.e., $F_K = \sqrt{\Sigma f_i^2}$, where the summation runs over all the enumerated experimental fractional errors and F_K is the resultant fractional error in the distribution coefficient. For bottom sediments the errors in K_d ranged from

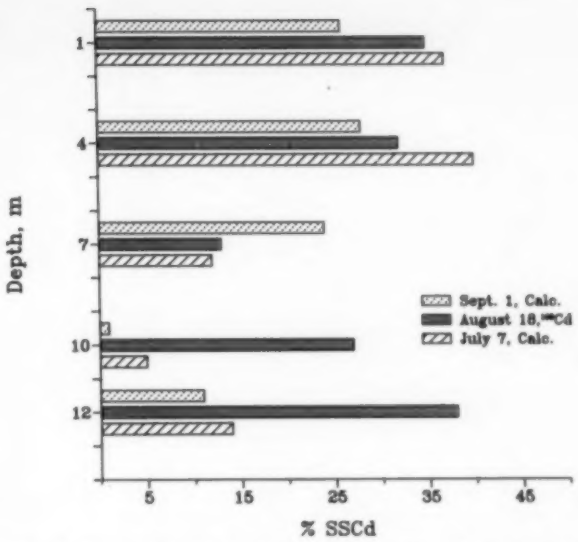

FIG. 1. Comparison of the calculated and measured fractions of Cd adsorbed on suspended particulate matter at 1, 4, 7, 10, and 12 m depths in Lake 382.

±2 to ±4% and for suspended matter ±12%, arising largely from the relatively large weighing errors in this case. For ^{109}Cd adsorbed on suspended matter in the lake (Lake 382) the counting errors in cpm for suspended matter/water were 9–21%/4% at 1–7 m depth and 31–35%/12–26% at >7 m depth. These relatively large counting errors were due to the decreasing concentration of ^{109}Cd with depth in the lake during the summer when the lake was stratified.

To be compatible with other constants in the speciation computer program MACS80 (Wagemann et al. 1990), K_d was redefined as K_c (eq. 2) in terms of appropriate concentration units (litres per milligram), and correction (a dimensionless factor) for complexion of Cd in solution by dissolved organic matter (humic acid), and the approximation 1 mL of water at 6°C = 1 g was used. K_c is related to K_d as follows:

$$(2) \quad K_c = K_d \times 10^{-6} \times \left(1 + \frac{f_{\mathrm{Cdorg}}}{f_{\mathrm{Cdfree}}} \right)$$

where f_{Cdorg} = fraction of total dissolved Cd bound to organic matter in solution and f_{Cdfree} = fraction of total dissolved Cd existing as "free" ionic Cd in solution; f_{Cdorg} and f_{Cdfree} were a function of pH, and the correction factor

$$(3) \quad \left(1 + \frac{f_{\mathrm{Cdorg}}}{f_{\mathrm{Cdfree}}} \right)$$

was calculated at each pH increment (Fig. 1) with the MACS80 chemical speciation computer program (Wagemann et al. 1990). In lake water of low ionic strength and relatively low pH (Lake 387), the effect of complexing by inorganic constituents on the K_c was negligible.

In June 1987, additions of Cd (up to 100 ng/L) with ^{109}Cd as a tracer (activity 1.0 Ci/g Cd) were made to Lake 382 in conjunction with biological experiments (Malley et al. 1989). This provided an opportunity for comparing the

TABLE 2. Parameters of the nonlinear regression of K_c on pH using the model $K_c = A \times 10^{B \times pH}$, K_c (L/mg), corrected for complexing by humic acid, and the corresponding K_d (L/g)[a], uncorrected) at pH 7.5, 7.0, and 6.5 for suspended particles and bottom sediments of Lake 382.

	Suspended particles	Bottom sediments O	Bottom sediments Q	Bottom sediments B
A	3.186×10^{-9}	2.303×10^{-6}	3.326×10^{-8}	1.318×10^{-11}
B	1.1593	0.6334	0.8645	1.1785
K_c (pH = 7.5)	1.58 ± 0.19	0.129 ± 0.005	0.101 ± 0.003	0.0091 ± .0002
K_d (uncorrected)	540 ± 65	80.5 ± 3.1	48.2 ± 1.4	2.80 ± 0.06
K_c (pH = 7.0)	0.415 ± 0.050	0.063 ± 0.002	0.037 ± 0.001	0.00230 ± 0.00004
K_d (uncorrected)	180 ± 22	39.4 ± 1.3	22.7 ± 0.6	1.49 ± 0.03
K_c (pH = 6.5)	0.109 ± 0.013	0.030 ± 0.001	0.0139 ± 0.0004	0.00060 ± 0.00001
K_d (uncorrected)	64.7 ± 7.7	20.3 ± 0.7	11.2 ± 0.3	0.91 ± 0.02

[a]To express K_d in units of L/mg, multiply given values by 10^{-3}.

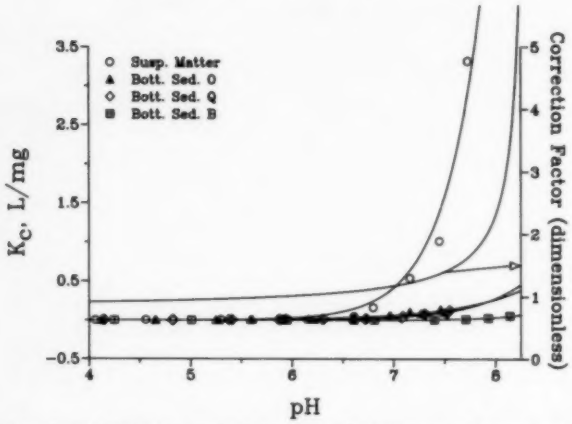

FIG. 2. Distribution coefficient (K_c) for bottom sediments and suspended particulate matter, and dissolved organic carbon correction factor for K_c's.

calculated and measured percent fractions of the total dissolved Cd associated with the suspended matter in the water. The fraction of Cd associated with suspended matter at different depths in the lake was measured (^{109}Cd) August 18, 1987. Calculations of the fraction of total Cd associated with suspended matter at depths of 1, 4 ,7, 10, and 12 m were made July 17 and September 1, 1987, bracketing the measurement date, with the MACS80 chemical speciation computer program using the K_c value for suspended matter and the other required measured inputs, primarily pH, dissolved organic matter, the major and minor ions, and the concentration of suspended particulate matter. Logistical contingencies precluded sampling for water chemistry parameters used in the speciation calculations on the same date as the sampling for ^{109}Cd on suspended matter. The total Cd concentration in the lake ($Cd_T = Cd^{2+} + Cd_{org} + Cd_{susp} +$ all Cd_{inorg}) had been brought up to 100 ng/L. The speciation program could account for over 300 different chemical species, including the interaction of metals with organic and suspended matter. The formation constant (Stevenson 1976) used for humic acid – Cd (2:1) complex was 6×10^{-4} (dimensionless), and the influence of ionic strength on the constant was accounted for in terms of a linear function of the square root of ionic strength. The Henderson–Hasselbalch equation with a pK_a value of 4.86 and a slope of −1.90 was used to calculate the ionization of the acid as a function of pH (Stevenson 1976). Other complex formation constants in the program were taken from a number of different sources, notably Berner (1967), Baes and Mesmier (1976), Smith and Martell (1976), and Nancollas et al. (1979). The program can print out the entire thermodynamic data base it uses, and the operational aspects of the program are described in the user's guide (Wagemann and Regehr 1992). The program calculates optimal "seed values" based on the input information for each system under consideration without the intervention of the operator, and the reiterative process seldom diverges. In the past, the calculations of the MACS80 program were compared with those of 14 other speciation programs for the same input data (Nordstrom et al. 1979). The MACS80 results were in agreement with those of the other programs.

Carbon in sediments was determined with the Perkin-Elmer 240 elemental analyzer which used thermal conductivity to detect CO_2. Dissolved organic carbon was determined with an IO Corp. model 700 automatic carbon analyzer which used IR to detect CO_2. This method compared favourably with three high-temperature catalytic oxidation methods (Hedges et al. 1993) with a relative precision of 5% among them. Silicon in sediments was determined by alkali fusion of the sediment sample and analysis by DCP-emission spectrometry (SMI IIIB). Other major ions in sediment were analyzed by flame atomic absorption with prior acid digestion of the sample. Methods for measuring major and some minor anions and cations in the water have been described (Stainton et al. 1974).

Total dissolved sulfide was determined by the methylene-blue method (American Public Health Association 1975) up to the point of separation of suspended solids ($Al(OH)_3$ floc) and colour development in the field, followed by spectrophotometric measurements (625 nm) in the laboratory. Tests showed that preparing the sample to the point of colour development in the field immediately after sampling was important in preventing loss of H_2S.

Results

The sediment–water distribution coefficients for suspended and bottom sediments for Cd in lake water were found to be strongly pH dependent at pH > 6.5 (Fig. 2):

(4) $K_c = A \times 10^{B \times pH}$.

For the three bottom sediments their somewhat differing carbon and silicon content (Table 1) had apparently no

TABLE 3. Equations governing the three major chemical Cd species in the water column.

Chemical equation	Remarks
$\frac{[A^-]}{[HA]} = 10^{\frac{4.86-pH}{1.9}}$	"A^-" and "HA" are the ionized and unionized humic acid concentrations, respectively (Stevenson 1976)
$\frac{[CdA_2]}{[HA]^2 \times [Cd]} = 6.4 \times 10^{-4}$	Constant (Stevenson 1976) depends on ionic strength ($\sqrt{I}$); this was taken into account by the MACS80 program
$\frac{[SSCd]}{[SSH_2]} = A \times 10^{B \times pH} \times [Cd]$	"SSCd" and "SSH_2" are Cd on suspended particles and the suspended particle concentration, respectively; A and B were obtained by curve fitting to experimental data

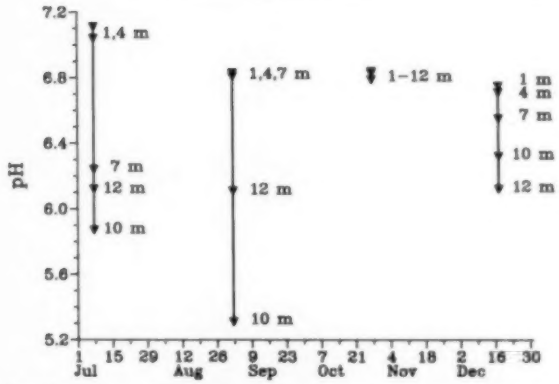

FIG. 3. pH at depths of 1, 4, 7, 10, and 12 m in Lake 382 at different times of the year.

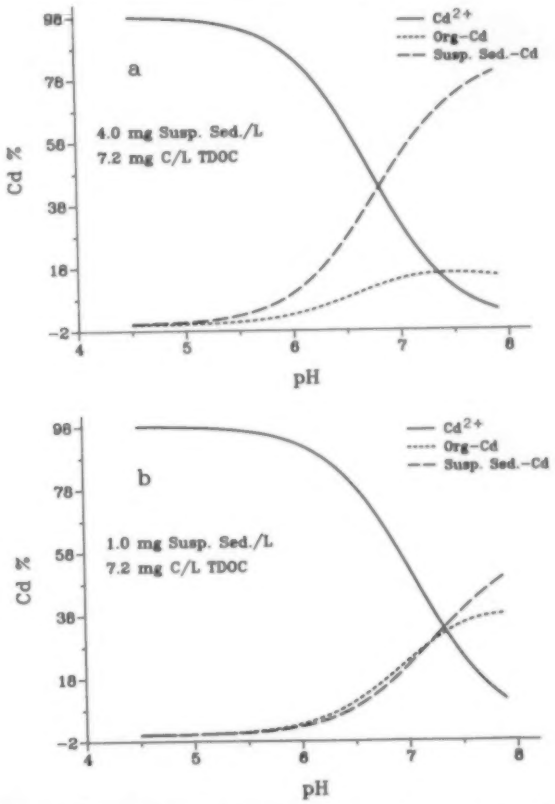

FIG. 4. Calculated percent fraction of total Cd in the water column as "free" Cd and Cd associated with (a) 4 mg suspended particulate matter/L and (b) 1 mg suspended particulate matter/L and with dissolved organic matter in Lake 382 as a function of pH, under aerobic conditions.

systematic influence on the K_c values. The exponential function (4) fitted very well all the K_c values for the bottom sediments and suspended matter. The numerical values A and B differed for different sediments ($p \leq 0.05$) (Table 2). For the three bottom sediments the K_c values were all <1 and differed from each other significantly (Table 2). For suspended matter the K_c values exceeded the bottom sediment values by an order of magnitude or more at neutral or alkaline pH, ranging from 0.109 to 1.58 L/mg for suspended matter and from 0.0006 to 0.129 L/mg for bottom sediments in the pH range $6.5 \leq pH \leq 7.5$. The concentration of Fe and Mn in suspended particles (in mid-August) was significantly higher in the hypolimnion than the epilimnion (Table 1), which may indicate that the suspended particulate matter distribution coefficient is a function not only of pH but also of depth. The distribution coefficients (K_c) reported here have been corrected for dissolved organic matter in the lake water as a function of pH (Fig. 2), i.e., they are expressed on the basis of zero concentration of dissolved organic matter and their pH dependency was calculated by exponential curve fitting to the data.

The subsequent additions of ^{109}Cd and stable Cd to Lake 382 (maintained at approximately 100 ng/L until 1990) in connection with impact studies on biota was used to determine experimentally the fraction of Cd adsorbed on suspended particulate matter in the lake as a function of depth (Fig. 1, middle bar). The experimentally determined suspended sediment distribution coefficient for Lake 382 was used (epilimnetic suspended particles) in the chemical speciation program (MACS80) to calculate the adsorbed fraction under varying conditions of pH and concentrations of water constituents at different depths in the lake. The measured (^{109}Cd) results were in agreement with the computed values down to a depth of 7 m (Fig. 1), although there was some difference in the calculated fractions on the two different dates, a consequence of changing conditions in the lake, particularly pH (Fig. 3), during the time interval the samples were taken. In deeper more acidic water the measured fraction exceeded the computed fraction.

Although the speciation program took into account many interactions between ions, under aerobic conditions in the absence of dissolved sulfide (epilimnion) the speciation

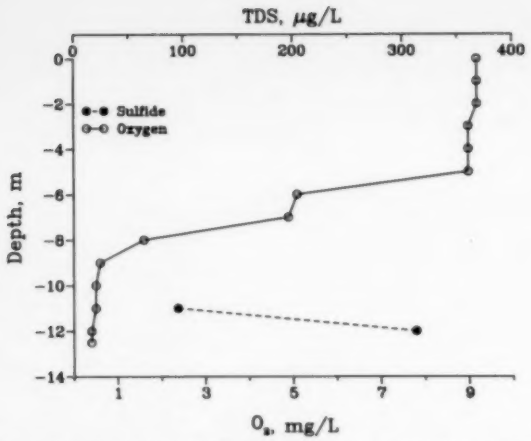

FIG. 5. Oxygen and total dissolved sulfide as a function of depth in Lake 382 on September 1, 1992.

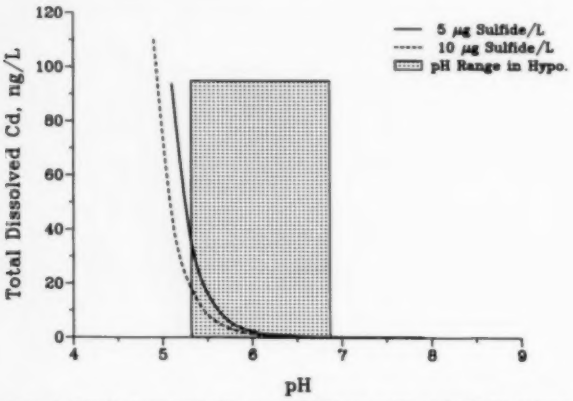

FIG. 6. Total dissolved Cd in equilibrium with solid CdS (given by *y*-coordinates of each curve within the shaded region) as a function of pH at 5 and 10 μg total dissolved sulfide/L. The *x*-coordinates (within shaded region) define the approximate pH range in the hypolimnion in Lake 382 in early September. In the area above each curve (within the shaded region), solid CdS formation can potentially occur.

calculations indicated that the main Cd species in lake water were "free" Cd, organically bound Cd, and suspended particle bound Cd, governed largely by the three relevant equilibrium equations (Table 3). The association of Cd with suspended particles and dissolved organic carbon was strongly pH dependent (Fig. 4a), the "free" Cd predominating at acidic pH, and the particle-bound and dissolved organically bound fractions at basic pH. At pH ≥ 7.5, essentially >85% of Cd in the water column was predicted to be associated with suspended particles and dissolved organic matter. This was confirmed by measurement of the particulate fraction in the epilimnion. Changes in the concentration of suspended particulate matter and dissolved organic carbon strongly affected the relative amount of Cd associated with each of these constituents. Calculations showed that a decrease from 4 to 1 mg suspended particulate matter/L (Fig. 4b) would decrease the Cd on particles from 72 to 39% and increase the organically bound Cd fraction from 16 to 35% and "free" Cd from 12 to 26% at pH 7.5.

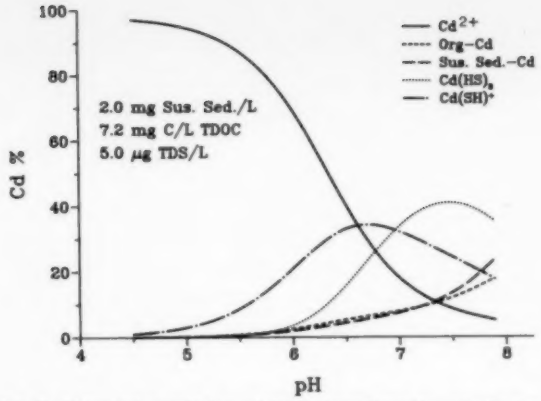

FIG. 7. Chemical speciation of Cd in hypolimnetic, anoxic water of Lake 382 in the presence of 5 μg dissolved sulfide/L.

In the hypolimnion the water was more acidic by as much as 1.5 pH units compared with the surface water (Fig. 3), and the calculated fraction of Cd associated with suspended particles in the deeper water was relatively low, only 2–14%, depending on depth and time. However, the measured amount of Cd on suspended particles significantly exceeded the calculated amount in acidic, hypolimnetic water. Sulfide was measured in the summer (1991, 1992) in Lake 382, and concentrations even near the bottom were <10 μg/L, but in September (1992), sulfide was relatively high in the deepest water where anoxic conditions prevailed (Fig. 5). Notwithstanding the acidic conditions in the lower part of the hypolimnion, significant CdS formation could have taken place there at that time of the year, and possibly earlier on. The control of dissolved Cd by solid CdS was investigated as a function of pH by speciation calculations (Fig. 6). In the acidic region the concentration of dissolved Cd allowed in the water in equilibrium with CdS was very sensitive to pH. Nevertheless, at the relatively high level of sulfide measured in the lower strata of the hypolimnion in early September, significant formation of CdS was certainly indicated, but could have also occurred in August when sulfide may have been present at a lower concentration (≤10 μg/L). The detection limit of the method used for measuring sulfide was 10 μg total dissolved sulfide/L. The CdS formed would be measured as part of the particulate fraction, since solid CdS, initially present as a colloidal suspension, would associate with other particulate matter upon filtration. This may have been the primary reason for the relatively high values obtained by measurement of Cd associated with the particulate fraction in the hypolimnion relative to the calculated values. Under anoxic conditions at neutral pH, any Cd remaining in solution would be relatively low (in the nanograms per litre range), even at a relatively low concentration of dissolved sulfide (5 μg/L), and would be largely in the form of sulfhydryl Cd species $Cd(HS)^+$ and $Cd(HS)_2$ (Fig. 7).

The possible control of Cd in Lake 382 by such solids as $CdCO_3$, $Cd(OH)_2$, and $Cd_3(PO_4)_2$ was also investigated as a function of pH by speciation calculations, which predicted that $Cd(OH)_2$ and $Cd_3(PO_4)_2$ would allow relatively high equilibrium concentrations of Cd in the water because of the low phosphate concentration and predominantly acidic nature of that lake. These anions would therefore not be of any consequence in the control of dissolved Cd in this

or similar oligotrophic Shield lakes. Because the water of Lake 382 was relatively low in inorganic carbon and acidic, carbonate would also fail to control the Cd concentration in this lake or other Shield lakes with similar water quality characteristics allowing relatively high dissolved Cd concentrations (tens of micrograms per litre) even at basic pH.

Discussion

Partitioning data for a number of different radioisotopes, but not Cd, between suspended matter and lake water have been published for a Shield lake (Schindler et al. 1980) and for Lake Constance (Sigg et al. 1982) for a number of metals, including Cd, unfortunately at an unspecified pH. Our distribution coefficient for bottom sediment O at pH 7.0, 63×10^{-3} L/mg is comparable with the value for Lake Constance sediment, 50 m^3/kg (50×10^{-3} L/mg), although this can obviously be only a rough comparison. For seawater and surficial sea bottom sediment, distribution coefficients have been determined at pH 7 for a number of radiotracers, including ^{109}Cd (Nyffeler et al. 1984), their values ranging from 47 to 95 L/kg for Cd (0.047×10^{-3} to 0.095×10^{-3} L/mg) for a number of collection sites. A strict comparison of the seawater K_d's with the values obtained for the bottom sediments of Lake 382 (Table 2) would require normalization of these constants to a common chloride concentration, since complexion of Cd by chloride in seawater is much more significant than in freshwater, as well as normalization to a common dissolved organic matter concentration; the latter information is generally unavailable post factum.

There were significant differences among the Cd distribution coefficients for the different bottom sediments from Lake 382, but any influence of composition of the sediments, notably organic matter and Si, on the partition coefficients was not apparent, possibly because of their narrow composition range. There were even greater differences between the coefficients for suspended particulate matter and bottom sediments. The difference in K_c's between these two types of sediment could be a manifestation of a particle concentration effect (Honeyman and Santschi 1988), or compositional differences, notably in Fe and Mn. Although sufficient suspended particulate matter was unavailable for a complete chemical analysis, the data obtained do indicate that Fe and Mn were significantly higher in hypolimnetic than in epilimnetic suspended particles, and Fe was significantly higher in bottom sediments than epilimnetic suspended particles (Table 1). The distribution coefficient for suspended particulate matter was much higher than for bottom sediments only in the alkaline pH range and depended strongly on pH (at pH > 6.5, Fig. 2). Mn and iron oxy/hydroxy compounds are well known, effective scavengers of trace metals which behave cyclically in their dissolution and precipitation in stratified lakes such as Lake 382. The differences among coefficients and their dependence on pH and possibly composition emphasize the need for judicious use of published distribution coefficients for modelling lacustrine systems. Ideally, the distribution coefficients for the system under consideration should be used to model it.

The calculations indicated that in a stratified lake such as Lake 382, less Cd would be adsorbed on suspended particles in the more acidic hypolimnetic water than the epilimnetic water, contrary to the experimental finding with ^{109}Cd. Some hypotheses can be advanced to account for this, the most likely one being the formation of CdS and its incorporation into the suspended particulate fraction. A relatively high concentration of H_2S was detected in early September in the most anoxic part of the hypolimnion of Lake 382 . Other lakes in this area are also known to generate H_2S during anoxic conditions in the range of 100–600 μg total dissolved sulfide/L and occasionally even higher (Prokopowich 1979; Linsey and Braund 1984; Linsey et al. 1985). The second hypothesis relates to the suspended particulate matter distribution coefficient itself, which was determined with epilimnetic suspended particles. Because the Fe and Mn concentrations differed greatly in hypolimnetic and epilimnetic suspended particles, the epilimnetic distribution coefficient may not have been strictly applicable to hypolimnetic suspended particles. The third hypothesis involves a possible lack of sorptive equilibrium in deeper water. In the summer, biological productivity is higher in the warmer epilimnion than the hypolimnion. The epilimnetic particles eventually reach the hypolimnion by settling. Calculated values always reflected an equilibrium situation, and ^{109}Cd epilimnetic particles may have been in sorptive equilibrium with their aquatic environment; however, ^{109}Cd measured on hypolimnetic particles may not have been in sorptive equilibrium if Cd became incorporated into living membranes during the time particles took to reach the lower strata. This too would overestimate the ^{109}Cd in the particulate fraction relative to the calculated adsorbed value. Sufficient time appears to have been available for such incorporation, since the settling velocity of particles was approximately 20 cm/d and the half-life for removal from the water column of a number of metal radioisotopes was 5–25 d in a similar Shield lake (Hesslein et al. 1980; Schindler et al. 1980). Lacustrine systems are clearly much more difficult to model than other more homogenous aquatic systems where concentration gradients and cyclical variations of chemical constituents are minimal or absent.

The calculated speciation results showed that under aerobic conditions, Cd in Lake 382 and in freshwaters of similar water quality would be present largely associated with suspended particles and dissolved organic matter at pH > 7.5 and very little as "free" Cd. This was confirmed by measurement of the adsorbed fraction in epilimnetic water. At pH < 6, nearly all Cd would be present as "free" Cd, unless the suspended matter concentration or dissolved organic matter were very much higher than generally found in such lakes. The speciation was quite sensitive to the concentration of suspended matter and pH in the neutral and alkaline pH region. Although the Cd concentration in Lake 382 was artificially raised to a relatively high level (100 ng/L up to 1989, 200 ng/L thereafter), solid $Cd(OH)_2$, $Cd_3(PO_4)_2$, or $Cd(CO_3$ could not form and thereby reduce the dissolved Cd concentration in this lake or other oligotrophic, softwater Shield lakes; however, dissolved sulfide could precipitate Cd. The sulfate ion itself complexes only weakly any cations present in freshwater and as such has little effect on the distribution of other chemical species except when it is reduced to sulfide.

Under anoxic conditions, even the relatively low concentration of 5 μg total dissolved sulfide/L would have sufficed to decrease the dissolved Cd concentration in the lower strata of the hypolimnion (forming CdS) to approximately 40–50 ng/L even in the most acidic stratum of the hypolimnion (pH 5.3) and certainly to much lower levels

at neutral and basic pH (Fig. 6) and act as an efficient scavenger of Cd. In moderately acidic or somewhat basic water, any dissolved Cd still remaining in the water column would be present largely in the form of two sulfhydryl species, $Cd(SH)^+$ and $Cd(HS)_2$ (Fig. 7).

In the short term, Cd is precipitated and remobilized into the water column cyclically, since sulfide appears and disappears cyclically in a dimictic lake such as Lake 382, in response to periodic oxic/anoxic conditions in the hypolimnion at spring and autumn "turnover". With each cycle, however, permanent burial of some of the precipitated CdS under sediment, natural flushing of the lake, and incorporation of Cd by biota and their eventual incorporation into sediments would reduce Cd to premanipulation natural background levels. Of the Cd solids investigated, CdS alone imposes the lowest concentration limit on dissolved Cd in the lake.

Acknowledgements

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Organization of Microsatellites Differs between Mammals and Cold-water Teleost Fishes

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Microsatellites, in particular $(dG\text{-}dT)_n$ and $(dG\text{-}dA)_n$ dinucleotide repeats, are abundant and display a high degree of length polymorphism and heterozygosity in eukaryotic genomes. Here, we report the cloning and characterization of 64 microsatellite sequences from Atlantic cod, *Gadus morhua*. The microsatellites were classified as perfect, imperfect, and compound repeats. The length and integrity of these repeats were compared with microsatellites characterized from two other teleosts, rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*), and from three mammalian genomes, human, porcine, and canine. Differences were found in the proportions of the repeat classes; however, the most significant difference between microsatellites from teleost fishes and mammals was the propensity of the former to be of greater length: some cod and rainbow trout microsatellites were more than twice the size of the longest microsatellite repeats reported for any mammalian genome. Primers for PCR amplification were constructed for seven of the cod microsatellites. Allele frequencies, degree of polymorphism, and heterozygosity were estimated for a sample population. Amplification with these cod primers was also carried out on a number of related gadids. These polymorphic microsatellite loci have enormous potential utility as genetic markers for use in population, breeding, and evolutionary studies.

Les chaînes d'ADN microsatellite, et plus particulièrement les séquences répétitives des dinucléotides $(dG\text{-}dT)_n$et $(dG\text{-}dA)_n$, sont abondantes et leurs génomes eucaryotes présentent un taux élevé de polymorphisme et d'hétérozygosité. Dans ce rapport, nous présentons les résultats du clonage et de la caractérisation de 60 séquences d'ADN microsatellite de la morue franche, *Gadus morhua*. Les ADN microsatellites étaient classées en trois catégories : parfaites, imparfaites et à séquence de répétition composée. La longueur et l'intégrité de ces séquences de répétition ont été comparées à celles d'ADN microsatellite caractérisée chez deux autres téléostéens, la truite arc-en-ciel (*Oncorhynchus mykiss*) et le saumon de l'Atlantique (*Salmo salar*), et chez trois génomes de mammifères, (humain, porcin et canin). On a trouvé des différences dans les proportions des catégories de séquences de répétition; toutefois, la différence la plus significative entre les ADN microsatellites de téléostéens et celle de mammifères était une tendance à une longueur supérieure chez ces derniers : les ADN microsatellites de certaines morues et truites arc-en-ciel étaient deux fois plus longues que les séquences répétées d'ADN microsatellite observées pour tous les génomes de mammifères. Des amorces pour l'amplification par la PCR ont été créées pour sept des ADN microsatellites de morue. Les fréquences d'allèles, le degré de polymorphisme et l'hétérozygosité ont été estimés pour une population échantillon. On a également fait l'amplification de ces amorces de morue pour un certain number de gadidés connexes. Ces locus d'ADN microsatellites polymorphes peuvent être extrêmement utiles comme marqueurs génétiques dans les études de population, de reproduction et d'évolution.

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Atlantic cod, *Gadus morhua*, is one of the most commercially important fish in the North Atlantic. Highly polymorphic genetic markers assayable via the polymerase chain reaction (PCR) hold the potential for considerable utility in selective breeding, fisheries management, and genome mapping of cod (Bentzen et al. 1991; Wright 1993; Wright and Bentzen 1994). To this end, we have cloned and sequenced microsatellite repeats from cod.

Microsatellites consist of tandem repeats of simple sequence DNA, where the varying number of repeats at a particular locus creates a polymorphism (Litt and Luty 1989; Tautz 1989; Weber and May 1989). These arrays of differing lengths can be amplified by PCR and size fractionated on acrylamide gels. The most abundant and widely used of these simple repeats are the dinucleotide sequences $(dG\text{-}dT)_n$ and $(dG\text{-}dA)_n$. Cloning of these microsatellites has been reported for a variety of species, although mostly mammals (Stallings et al. 1991). At present, extensive microsatellite data for human (Weber 1990), mouse (Love et al. 1990), rat (Beckmann and Weber 1992), dog (Ostrander et al. 1993), pig (Wintero et al. 1992), horse (Ellegren et al. 1992), bees (Estoup et al. 1993), Atlantic salmon (*Salmo salar*) (Slettan et al. 1993), brown trout (*Salmo trutta*) (Estoup et al. 1993), and zebrafish (Goff et al. 1992) have been published.

Highly polymorphic microsatellite markers have great potential utility as genetic tags for use in aquaculture and

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TABLE 1. Repeat sequence type and size of microsatellites from mammalian and teleost fish species. Figures in parentheses are the second highest size class due to a bimodal distribution.

	Atlantic cod	Rainbow trout	Atlantic salmon	Human	Canine	Porcine
n	64	51	45	114	101	105
% perfect	48.4	56.9	80	64	80	71
% imperfect	45.3	31.4	20	25	8	19
% compound	6.3	11.7	0	11	12	10
Most common size class[a]	6–11 (30–35)	24–29	6–9 (21–24)	12–15	14–16	16–18
Largest size class[a]	>60	>60	>33	27–30	23–25	28–30

[a]Number of dinucleotide repeats.

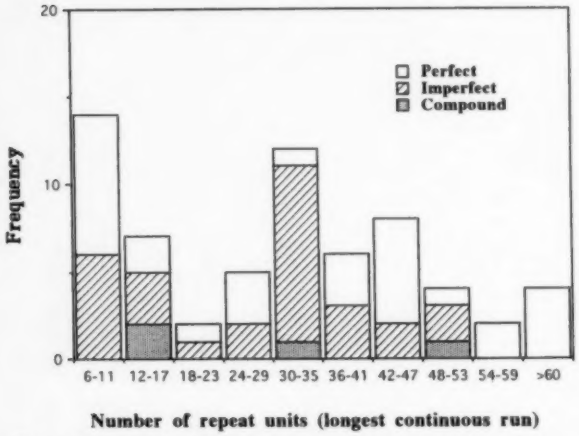

FIG. 1. Frequency of different size classes of the longest uninterrupted microsatellite arrays from Atlantic cod. Size classes are represented as number of repeat units.

fisheries biology. They may prove particularly valuable for stock discrimination and population genetics due to the high levels of polymorphism compared with conventional allozyme markers (Bentzen et al. 1991; Wright and Bentzen 1994). The utility of the PCR approach also makes possible the processing of large sample numbers required for population surveys and large-scale breeding programs. Moreover, the minute amount of tissue required to genotype individuals allows larvae as well as retrospective studies of archival samples such as otoliths to be examined (Wright and Bentzen 1994).

Recently, microsatellites from Atlantic salmon were reported to differ significantly in length and composition from mammalian microsatellites (Slettan et al. 1993). In order to determine if this finding is a general trend for microsatellites isolated from teleost fishes, we have investigated the organization of Atlantic cod and, to a lesser extent, rainbow trout (*Oncorhynchus mykiss*) microsatellites and compared them with microsatellites isolated from other species. We also report the allele distributions and heterozygosities of seven microsatellite loci that exhibit a wide range of polymorphism in a sample of 127 cod from the western North Atlantic Ocean and investigate whether or not these microsatellite loci are conserved in related gadid species.

TABLE 2. Nucleotide sequence of Atlantic cod microsatellites and primers for amplification of loci by PCR.

Primer	Sequence 5′ → 3′	T_{ann} (C)[c]
Gmo1	$(GA)_4N(GA)_2N_2(GA)_7N_2(GA)_4N_2(GA)_5$	57
F[a]	AAA AAC AAA ACG AGA GCG GT	
R[b]	GGA GTT GCT TGT GGT GGC AT	
Gmo2	$(GT)_{14}$	51
F[a]	CCC TCA GAT TCA AAT GAA GGA	
R[b]	GTG TGA GAT GAC TGT GTC G	
Gmo9	$(GT)_6N_2GTN_2GTN_2(GT)_{46}$	50
F	GCA GAT TTG AAT CAG CGT GT	
R	CTC TGT GGG TAT GTG CAA AG	
Gmo10	$(GT)_5N_2(GT)_3N_2(GT)_2N_2(GT)_{44}$	53
F	GGA GTT TGA CTC ATC AGA TGC	
R	ACA GCG TAT GAC CAT GAT TCG	
Gmo123	$(GT)_8N_2(GT)_{11}N(GT)_2N_3(GT)_{43}N_3(GT)_8$	56
F	GAG GGA CAT AAA GAC ACT T	
R	AAA CAT GCA TGC AGG CAA C	
Gmo132	$(GT)_{19}$	52
F	GGA ACC CAT TGG ATT CAG GC	
R	CGA AAG GAC GAG CCA ATA AC	
Gmo145	$G_{12}(GA)_{29}$	50
F	GCA TTG TAG GAA CAA CAA TTA AC	
R	GTG CAT GTG CTC ATT ATA GC	

[a]*F* = forward primer.
[b]*R* = reverse primer
[c]Annealing temperature.

Methods and Materials

Isolation of Microsatellites from Cod

Two cod genomic libraries were constructed. The first employed the vector M13mp9 and was constructed from purified, size-selected fragments of 200–500 bp. These fragments were generated by digestion with enzymes *Alu*I, *Hae*III, *Hin*cII, and *Rsa*I (Pharmacia) according to the manufacturer's instructions and cloned into the *Sma*I site of the vector. Construction of a second library was performed by cleaving cod genomic DNA with *Alu*I, *Hin*cII, *Pal*I, and *Rsa*I, followed by ligation of size-selected fragments of 300–800 bp into the *Sma*I site of the pUC18 plasmid.

TABLE 3. Pedigree analysis of three two-generation families of Atlantic cod using six polymorphic microsatellite loci.

Fish	Gmo1	Gmo2	Gmo9	Gmo123	Gmo132	Gmo145
AI_1	1, 1	3, 4	4, 7	1, 4	4, 5	2, 8
AI_2	1, 1	2, 5	8, 9	2, 5	4, 5	5, 10
AII_1	1, 1	2, 4	4, 8	4, 5	4, 4	2, 10
AII_2	1, 1	3, 5	4, 9	1, 5	5, 5	2, 10
AII_3	1, 1	2, 3	7, 9	4, 5	4, 5	8, 10
AII_4	1, 1	2, 4	4, 9	1, 2	4, 4	8, 10
AII_5	1, 1	3, 5	4, 9	2, 4	5, 5	5, 8
AII_6	1, 1	2, 4	4, 9	2, 4	4, 4	2, 5
AII_7	1, 1	2, 4	4, 8	4, 5	4, 4	2, 10
AII_8	1, 1	3, 5	4, 9	4, 5	5, 5	8, 10
AII_9	1, 1	2, 3	7, 8	1, 5	5, 5	2, 10
AII_{10}	1, 1	4, 5	7, 9	2, 4	4, 5	5, 8
BI_1	1, 1	2, 3	2, 3	6, 10	1, 2	4, 7
BI_2	1, 1	1, 5	6, 6	2, 4	3, 5	3, 9
BII_1	1, 1	3, 5	3, 6	2, 10	1, 5	4, 9
BII_2	1, 1	1, 3	3, 6	2, 10	1, 3	7, 9
BII_3	1, 1	3, 5	3, 6	2, 10	2, 5	4, 9
BII_4	1, 1	1, 2	2, 6	2, 6	1, 5	4, 9
BII_5	1, 1	2, 5	3, 6	4, 10	2, 3	4, 9
BII_6	1, 1	1, 3	3, 6	4, 10	1, 5	3, 7
CI_1	1, 1	2, 5	1, 8	7, 9	5, 5	1, 10
CI_2	1, 1	2, 5	5, 7	3, 8	3, 5	5, 6
CII_1	1, 1	2, 5	5, 8	3, 7	3, 5	1, 5
CII_2	1, 1	2, 2	1, 7	8, 9	5, 5	1, 5
CII_3	1, 1	2, 5	7, 8	3, 7	5, 5	1, 5
CII_4	1, 1	2, 2	7, 8	3, 9	5, 5	1, 6
CII_5	1, 1	2, 5	1, 5	3, 9	5, 5	1, 6
CII_6	1, 1	5, 5	1, 7	8, 9	5, 5	5, 10
CII_7	1, 1	5, 5	5, 8	7, 8	5, 5	6, 10
Size of alleles (bp)	1 = 104	1 = 116 2 = 114 3 = 112 4 = 110 5 = 108	1 = 236 2 = 220 3 = 216 4 = 212 5 = 208 6 = 206 7 = 202 8 = 194 9 = 190	1 = 221 2 = 219 3 = 213 4 = 203 5 = 201 6 = 199 7 = 195 8 = 191 9 = 187 10 = 141	1 = 135 2 = 123 3 = 119 4 = 113 5 = 111	1 = 183 2 = 181 3 = 179 4 = 177 5 = 175 6 = 173 7 = 171 8 = 169 9 = 167 10 = 165

Following transformation, bacterial colonies or plaques were transferred to nylon filters (Amersham) according to the manufacturer's instructions and the immobilized DNA was hybridized to $(GT)_{15}$ synthetic oligonucleotide, radiolabeled with [γ ^{32}P]ATP and T4 polynucleotide kinase. Filters were incubated in prehybridization solution of Westneat et al. (1988) at 65°C for 1 h before addition of the radiolabeled probe. The hybridization reaction was allowed to proceed overnight. Filters were washed once with 2 × SSC/0.2% SDS at room temperature for 15 min and once with 2 × SSC/0.2% SDS at 65°C for 15 min. Dideoxy-chain termination sequencing of DNA from purified positive clones was accomplished using T7 DNA polymerase (Pharmacia).

PCR Analysis

PCR primers complementary to the sequence flanking the microsatellite were synthesized. For all PCR analyses, except those that amplify the locus Gmo9, the reverse primer was 5′ end-labeled with [γ ^{32}P]ATP (37 kBq/10 pmol primer) using T4 polynucleotide kinase (Pharmacia). PCR was carried out in a Perkin Elmer thermal cycler using Taq Polymerase (Perkin Elmer). The standard reaction contained 10 ng of template DNA, 0.6 μM labeled reverse primer, 0.6 μM forward primer, 10 mM Tris–HCl (pH 8.3), 1 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin, 200 μM dNTPs, and 0.25 unit of Taq Polymerase in a 10-μL volume. For Gmo9, the reverse primer was not labeled; instead, [α ^{32}P]dATP was included in the PCR mix along with 20 mM $MgCl_2$. Amplifications were achieved by running 30 cycles of 1 min at 95°C, 2 min at the appropriate annealing temperature (see Table 2), and 20 s at 72°C. Stop dye (10 μL, Pharmacia) was added to each reaction and the products were denatured at 80°C for 5 min. Two microlitres of the reaction was subjected to gel electrophoresis on a 6% denaturing polyacrylamide gel.

Results and Discussion

Characterization of Cod-derived $(dG\text{-}dA)_n$ and $(dG\text{-}dT)_n$ Sequences

As a result of the two cloning efforts, over 120 clones

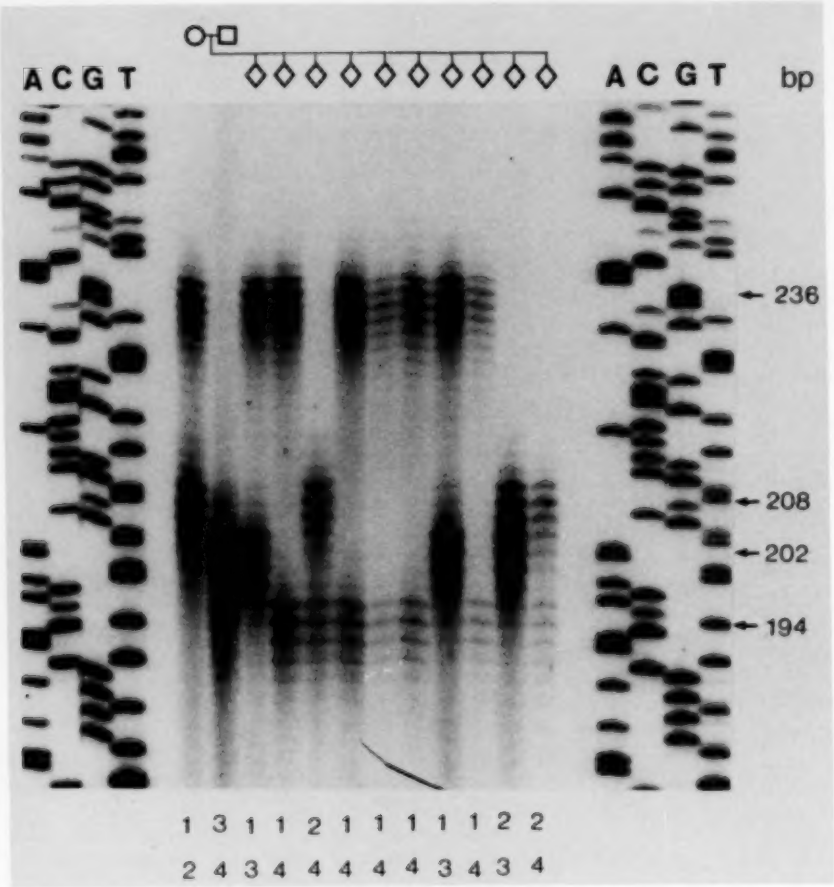

FIG. 2. Allelic products generated by PCR of the Gmo9 locus from a two-generation Norwegian pedigree of Atlantic cod. Offspring demonstrate stable inheritance of parental alleles which are numbered and shown at the bottom of the figure. Size of alleles are estimated using an M13 sequence ladder.

that hybridized to the dinucleotide probe were isolated and sequenced from Atlantic cod, approximately half from each library. A crude estimate of the number of times $(dG\text{-}dT)_n$ arrays occur in the genome and the distance between these arrays was calculated. A plasmid library of approximately 7000 clones containing an average insert size of 400 bp was constructed, representing 2.8×10^6 bp of cod genomic DNA. We make the assumption that $(GT)_n$ sequences are distributed evenly throughout the cod genome and also that the 54 positive clones that we sequenced were representative of all the positive clones (about 10%). Thirty-one of 54 clones, or 57%, contained a $(GT)_n$ array. Based on a "*c*" value of 0.9 pg, the haploid genome of cod is 821 Mbp (Hinegardner and Rosen 1972); we estimate that there are approximately 2.34×10^5 copies of $(GT)_n$ microsatellite sequence present in the cod genome at intervals of 7 kbp. These estimates are comparable with those suggested for mammalian genomes (Tautz 1989) and approximately three times more frequent than in brown trout (Estoup et al. 1993).

Forty-nine clones harbored repeats bracketed by nonrepetitive sequences. These were classified as perfect, imperfect, and compound according to Weber (1990). Briefly, perfect repeats are uninterrupted stretches of the repeat unit, while imperfect repeats have one to three intervening bases with repeat sequence on either side. Compound repeats consist of neighboring blocks of different types of repeat sequence.

Sixty-four independent microsatellite arrays were identified from the 49 clones. The longest perfect stretch of each of these arrays was plotted according to class and number of uninterrupted repeat units (Fig. 1). The percentage of each class of repeat, the most common size class, and the longest size class for cod were compared with microsatellites from other species: human (Weber 1990), pig (Wintero et al. 1992), dog (Ostrander et al. 1993), Atlantic salmon (Slettan et al. 1993), and rainbow trout (unpublished data) (Table 1).

Slightly different procedures have been used to isolate microsatellites from different species. It is possible that we may have biased our screening towards longer arrays with the use of a $(GT)_{15}$ oligonucleotide and higher hybridization and washing temperatures (65°C); however, we feel that this is unlikely. Less stringent screening efforts for mammals, as used by Weber (1990), Beckmann and Weber (1992), Ellegren et al. (1992), Wintero et al. (1992), and Ostrander et al. (1993) have failed to reveal microsatellites over 30 repeats

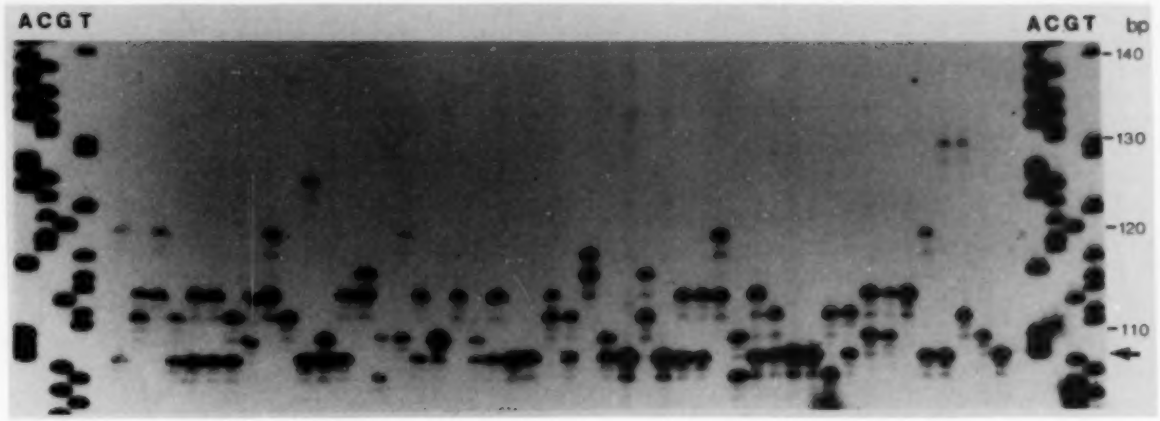

FIG. 3. PCR amplified alleles from 48 Atlantic cod individuals at microsatellite locus Gmo2. The sizes of the PCR products were estimated by co-migration with those of the M13 sequence fragments shown at either side of the cod samples.

in length. Screening at a lower stringency should enhance detection of long, short, and more degenerate dinucleotide arrays, but there is no evidence of this in the literature.

In all animal species, $(dG\text{-}dT)_n$ microsatellites predominate with the next abundant type being $(dG\text{-}dA)_n$. For this reason, we chose to focus our search on $(dG\text{-}dT)_n$. We only succeeded in isolating two $(dG\text{-}dA)_n$ microsatellites, detected by a $(GT)_n$ probe; these were found in clones containing a $(dG\text{-}dT)_n$ array. The proportions of the different classes of repeat types differ between species, with the highest percentage of imperfect repeats found in cod and rainbow trout. Proportions of perfect and imperfect microsatellites isolated from Atlantic salmon (Slettan et al. 1993) more closely resemble the mammalian genomes, except for the absence of compound repeats, than do microsatellites from Atlantic cod and rainbow trout (Table 1). An even more striking difference between mammalian and cold-water fish microsatellites is the most common size class of the microsatellite arrays. Sixty-four percent of the smallest class of arrays (containing 6–11 repeats) in cod were separated by four or five bases adjacent to a larger repeat in the same clone. They were classified as separate repeats because of the arbitrary definition of Weber (1990). If these shorter microsatellites are viewed as part of a larger degenerate microsatellite array, and the criteria which define an imperfect repeat are altered to incorporate four or five intervening bases rather than three, then the most common size class for cod microsatellites would be at least 30–35 repeats, approximately twice the size of those reported in mammalian genomes. Regardless of this, the next abundant size classes for cod and Atlantic salmon are 30–35 and 21–24 dinucleotide repeats, respectively, significantly larger than the most common size classes reported for mammals. This conclusion is consistent with the size of microsatellites found in rainbow trout (unpublished data), where smaller adjacent repeats were not found.

The absolute lengths of the largest microsatellite arrays in cod, rainbow trout, and Atlantic salmon (Slettan et al. 1993) are also greater than those reported in mammalian genomes. In a study of microsatellite arrays from zebrafish, although not providing data on all repeat arrays, three of 16 sequences were over 30 repeat units in length, the longest consisting of 38 dinucleotide units (Goff et al. 1992). Data were statistically analyzed to see if this difference in microsatellite length was significant for fishes and mammals. A chi-squared test was inappropriate because of the varying size classes used by the different authors to group data from the different species. A new data set was constructed by taking the midpoint of the microsatellite length of each size class and then weighting the length data by frequency of the class. Bartlett's test for homogeneity of variance showed that the fish data were more variable than the mammal data ($P < 0.001$). A Kruskal–Wallis nonparametric analysis on the pooled mammal and fish data demonstrated that the maximum lengths of fish and mammalian microsatellites are highly different ($P < 0.001$).

TABLE 4. Allele size and heterozygosity at Atlantic cod microsatellite loci detected by PCR amplification.

Locus	No. of alleles	Size (bp)[a]	% heterozygosity
Gmo1	8	94–114	14
Gmo2	15	106–140	82
Gmo9	38	116–246	92
Gmo10	1	214	0
Gmo123	46	137–239	89
Gmo132	15	109–155	85
Gmo145	26	123–217	92

[a]Estimated size of the PCR fragment when compared with M13 sequence fragments of known length.

Also noteworthy, the longest microsatellite arrays in the cod genome consisted of perfect dinucleotide repeats. In rainbow trout, six of seven microsatellites longer than 47 repeat units were perfect motifs. Cod, rainbow trout, and possibly Atlantic salmon and zebrafish microsatellites are longer and more degenerate than similar mammalian sequences. Cod and rainbow trout microsatellites are similar in length, but there is a higher proportion of imperfect repeats in the cod, often with smaller degenerate repeats adjacent to larger ones.

Inheritance and Allele Frequencies at Seven Microsatellite Loci

PCR primers were designed from flanking regions for a number of microsatellites and conditions such as temperature,

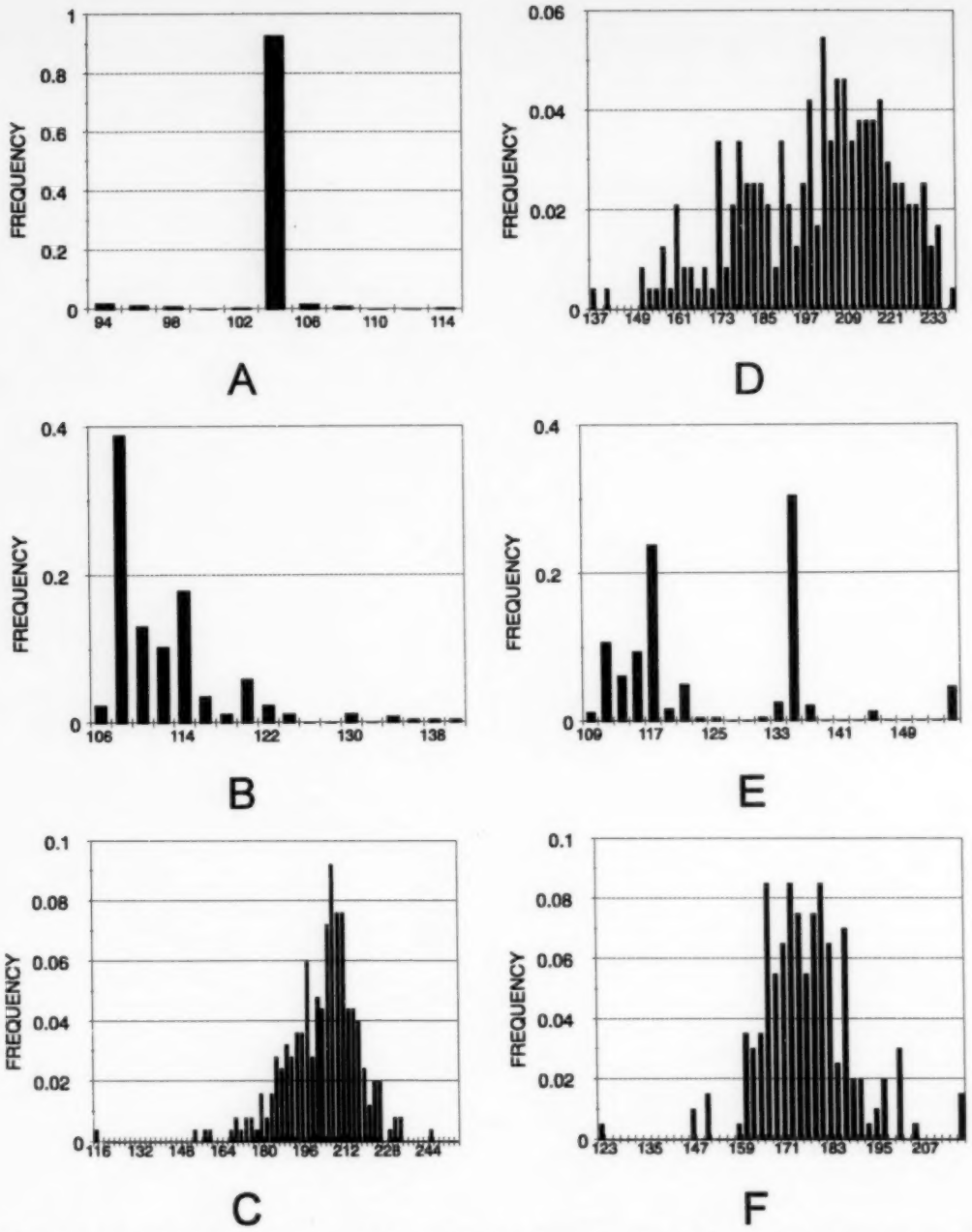

FIG. 4. Allele frequency distribution of alleles at microsatellite loci from Atlantic cod from the Northwest Atlantic Ocean (n = 127). Microsatellite loci: (A) Gmo1, (B) Gmo2, (C) Gmo9, (D) Gmo123, (E) Gmo132, and (F) Gmo145.

radiolabeling (end labeling versus incorporation), and magnesium concentration were optimized (Table 2). Seven primer pairs produced a product readily scored. All seven loci exhibited stable inheritance of alleles by PCR assay in three unrelated, two-generation pedigrees of Atlantic cod (Table 3). Figure 2 demonstrates that even the most complex fragments produced by PCR of the Gmo9 locus can be readily scored.

Genomic DNA samples from 127 fish caught off the coast of Nova Scotia in the western North Atlantic were used to amplify seven microsatellites with the primer pairs described in Table 2. Alleles for each individual were scored by comparing their migration with sequence fragments from M13 of known length (Fig. 3). The seven microsatellites varied widely in the degree of polymorphism exhibited (Table 4). Of the seven microsatellites, six were polymorphic, with 8–46 alleles and heterozygosities of 14–92%. Five of the microsatellites exhibited heterozygous arrays in excess of 80%. It is interesting that the only locus which was monomorphic

(Gmo10; $(GT)_5N_2(GT)_3N_2(GT)_2N_2(GT)_{44}$ consists of a long imperfect repeat, while all of the short repeats such as Gmo2, Gmo132, and Gmo145 were highly polymorphic. This result suggests that factors other than overall length of a microsatellite array, as suggested by Weber (1990), may determine whether a microsatellite will exhibit allelic variation (see Wright 1993).

The allele frequency distributions varied markedly for the seven microsatellite loci assayed (Fig. 4; Table 4). Some microsatellites showed wide variation in allele size (e.g., Gmo9 and Gmo123). Most allele distributions were unimodal, but those of Gmo9 and Gmo132 appeared weakly and strongly bimodal), respectively (Fig. 4; Table 4). Similar allele frequency distributions, reported for human microsatellites (Valdes et al. 1993), have been attributed to a stepwise mechanism for the generation of new alleles. Presumably, a similar mechanism is probably responsible for the generation of allelic variation at microsatellite loci in cod.

In order to determine the evolutionary conservation of microsatellite loci in related gadids, PCR amplification was attempted with the seven primer pairs for the Atlantic cod microsatellite loci on genomic DNA from the following species: haddock (*Melanogrammus aeglefinus*), pollock (*Pollachius virens*), and white hake (*Urophycis tenuis*). PCR products were detected in all of the gadid samples for the loci Gmo1, Gmo2, and Gmo9, while Gmo10, Gmo132, and Gmo145 produced fragments in only haddock and pollock. No products were detected after PCR with the Gmo123 primers. Although only three individuals for each species were assayed, all of the loci except Gmo10 and Gmo123 appeared to be polymorphic for at least one of the other gadids (data not shown).

General Discussion

We report here the characterization of a group of microsatellites from *Gadus morhua*. It appears that the microsatellites in teleost fishes differ significantly in length and composition from those of mammals. We can only speculate as to why the microsatellites found in Atlantic cod, rainbow trout, and Atlantic salmon are larger than microsatellites from mammals. It remains to be seen, with subsequent isolation of microsatellites from other species, whether poikilotherms consistently have longer arrays than homiothermic species. The longer, degenerate microsatellite arrays found in teleost fishes may be caused by a predisposition to slippage owing to fluctuations in temperature. The DNA polymerase may not function efficiently when subjected to changes in temperature of several degrees, or temperatures close to freezing, such as occur in the Atlantic cod environment and that of the salmonid species.

Disparities in genomic composition between warm- and cold-blooded vertebrates have already been established by Bernardi and Bernardi (1990). They found that fish isochores were much more homogeneous and lower in GC content than those of warm-blooded vertebrates. A subsequent study by Cross et al. (1991) makes a further distinction between fish genomes and other warm- and cold-blooded vertebrates with regard to the GC-poor CpG islands that are associated with some genes. It is possible that different evolutionary forces are acting on the fish genome, generating longer repeat arrays at microsatellite loci, or conversely, shorter arrays in the genomes of mammals.

Using PCR and primers specific to the nonrepetitive flanks, we have successfully amplified from cod genomic DNA seven of the cod microsatellites isolated in this study. Five of these microsatellites exhibit heterozygosities greatly in excess of those reported for allozyme or mitochondrial studies. The average heterozygosity of these microsatellite markers is $H = 0.85$ compared with $H = 0.071$ for allozymes (Mork et al. 1985) and an equivalent measure for nucleon diversity, $h = 0.36$, for mitochondrial genotypes (Carr and Marshall 1991).

Breeding and population studies of Atlantic cod utilizing these microsatellites as genetic markers are in progress in our laboratory. The family Gadidae to which Atlantic cod belong includes many other economically important species. Our results have shown that the microsatellites reported here may prove useful as genetic markers in other gadid species, similar to a study previously carried out on domestic mammals (Moore et al. 1991). Furthermore, a comparative study of the conservation and polymorphism of these microsatellite loci in related gadids may elucidate the mode and tempo of mutation at these fascinating sequences (Bentzen and Wright 1993; Wright 1993, 1994).

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Sublethal Effects of Tetrachloroguaiacol on Juvenile Rainbow Trout, *Oncorhynchus mykiss*, following Acute and Chronic Exposure

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Following either a 24-h (acute) or 25-d (chronic) exposure to tetrachloroguaiacol (TeCG), a component of bleached kraft mill effluent (BKME), juvenile rainbow trout, *Oncorhynchus mykiss*, were examined for effects on several biochemical parameters, swimming performance, and disease resistance. Acute exposure resulted in increased levels of plasma cortisol and lactate and reductions in liver glycogen and protein, spleen somatic index, leucocrit, hemoglobin, swimming performance, and disease resistance. Chronic exposure resulted in most parameters remaining at control levels with the exception of leucocrit which was elevated, plasma cortisol which decreased, and disease resistance which was impaired. These findings correlate well with a previously proposed classic stress response for mammals and are generally consistent with studies in which fish were exposed to whole BKME. The 96-h LC_{50} for juvenile rainbow trout exposed to TeCG was estimated at 0.37 $mg \cdot L^{-1}$.

Après une exposition de 24 h (aiguë) ou de 25 d (chronique) au tétrachloroguaiacol (TeCG), qu'on retrouve dans les effluents de la pâte kraft blanchie (EPKB), on a examiné des truites arc-en-ciel, *Oncorhynchus mykiss*, juvéniles afin de déceler des effets de l'exposition sur plusieurs paramètres biochimiques, l'aptitude à la nage et la résistance aux maladies. Les expositions aiguës ont entraîné des augmentations des concentrations de cortisol et de lactate plasmiques, ainsi que des réductions du glycogène et des protéines du foie, de l'indice somatique de la rate, de la leucocrite, de l'hémaglobine, de l'aptitude à la nage et de la résistance aux maladies. Dans le cas des expositions chroniques, la plupart des paramètres restaient inchangés par rapport aux valeurs des témoins, sauf la leucocrite, qui était plus élevée, le cortisol plasmique, qui était plus faible, et la résistance aux maladies, qui était diminuée. Ces constatations concordent assez bien avec les valeurs habituelles de réponse au stress déjà proposées pour les mammifères et sont généralement cohérentes avec des études de poissons exposés à des effluents entiers d'EPKB. La CL_{50}-96 h de la truite arc-en-ciel juvénile exposée au TeCG a été évaluée à 0,37 $mg \cdot L^{-1}$.

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Concern over the effect of pulpmill effluent on fish populations has resulted in numerous studies dealing with bleached kraft pulp mill effluent (BKME). However, the composition and toxicity of BKME vary greatly from site to site and even daily or hourly within the same mill (Oikari et al. 1984). Oikari et al. (1984, 1988) used a standardized, simulated effluent to eliminate this variability in their experiments. In this study, we chose to concentrate on one important component of BKME, tetrachloroguaiacol (TeCG).

Chloroguaiacols are chlorinated phenolic compounds formed from lignin residues during chlorine bleaching of wood pulps (Rosemarin et al. 1990). Leach and Thakore (1975) found that most of the toxicity of BKME to fish could be attributed to a fraction containing resin acid derivatives and chlorinated lignin breakdown products that include the chloroguaiacols. While effluent treatment using biological oxidation ponds is effective in detoxifying resin acids and other toxic wood extractives, this process is not efficient in removing chloroguaiacols (Rogers et al. 1988). Rosemarin et al. (1990) estimated that the amount of chloroguaiacol produced from a kraft mill is on the order of 1 $mg \cdot L^{-1}$ of effluent whereas trichloroguaiacol (TCG) and TeCG levels of 0.2–1.0 and 0.3 $\mu g \cdot L^{-1}$, respectively, have been found as far as 3 km downstream from the discharge point (Voss and Yunker 1983). In a survey of the Fraser River, Rogers et al. (1988) found tissue levels of TCG and TeCG in fish as

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high as 304 and 136 $\mu g \cdot kg^{-1}$, respectively. Schrier et al. (1991) suggested that the significant levels of TCG and TeCG found in fish of the lower Fraser River indicate that pulp mills can have potential impacts hundreds of kilometres downstream. Despite these findings, our knowledge of chloroguaiacol toxicity in fish is limited to few studies using acute lethality tests.

Of the chloroguaiacols 3,4,5,6-tetrachloro-2-methoxyphenol (TeCG) seems to be the most acutely toxic to fish (Renberg et al. 1980) with a 96-h LC_{50} in rainbow trout, *Oncorhynchus mykiss*, of approximately 0.32 $mg \cdot L^{-1}$ (Leach and Thakore 1977). In this study, we examined the sublethal effects of TeCG in juvenile rainbow trout following either an acute 24-h or a chronic 25-d exposure using a number of biochemical and physiological parameters that are biologically and ecologically relevant (Adams 1990).

Materials and Methods

Fish

Juvenile rainbow trout from a single year class and stock were obtained from West Creek Trout Farms, Aldergrove, B.C., and maintained at a temperature that varied seasonally (10–16°C) in 2000-L fiberglass tanks under natural photoperiod. The mean weight and fork length of the fish (±SEM) were 9.11 (0.61) g and 9.81 (0.79) cm, respectively. Fish were fed daily ad libitum with commercial salmon pellets (Moore-Clark Co. Inc., La Conner, Wash.). Filtered and dechlorinated municipal tap water with a pH of 6.1–6.3, hardness of 5.2–6.0 mg $CaCO_3 \cdot L^{-1}$, and dissolved O_2 content above 95% saturation was used throughout the study.

Chemicals, Exposure, and Analysis

TeCG (99%) was purchased from Helix Biotech Corp. (Richmond, B.C.) and dissolved in 4 mL of 100% ethanol and 4 mL of 1 N NaOH and added to 4 L of distilled water as a stock solution to a final concentration of 326 $mg \cdot L^{-1}$. Trout were exposed to TeCG using a flow-through bioassay system as described by Johansen and Geen (1990). A Transduction LSI 11/02 compatible computer using a custom-built interface and proprietary software allowed the investigators to generate different concentrations of TeCG in each glass aquarium (see below). Using a series of computer-controlled pumps and solenoid valves the appropriate amount of TeCG stock solution and fresh water was delivered to each aquarium every 30 min, replacing 175 L daily and maintaining the desired concentration. The TeCG delivery system was calibrated prior to the study with a series of dye tests using Trypan Blue. External disturbances were minimized by isolating individual treatment groups with black polyethylene screens. A 24-h acclimation period preceded the start of each trial and all trials were conducted according to bioassay guidelines outlined by Sprague (1973) and the American Public Health Association (1976).

Acute and Chronic Studies

In the 24-h acute exposures, nine fish were stocked in each of five 8-L glass aquaria receiving different nominal TeCG concentrations of 0, 0.1, 0.2, 0.3, and 0.4 $mg \cdot L^{-1}$. Immediately following the 24-h exposure, fish were either sacrificed for biochemical analysis or used for either swimming performance or disease resistance studies. The 24-h protocol was repeated four times to provide fish for each of the parameters examined.

For the 25-d chronic exposure, 50 fish were stocked in each of four 144-L glass aquaria receiving different nominal TeCG concentrations of 0, 0.02, 0.1, and 0.2 $mg \cdot L^{-1}$. Immediately following the 25-d exposure, fish in each treatment group were divided up randomly and either sacrificed for biochemical analysis or transferred for either swimming performance or disease resistance studies.

Biochemical Analysis

After both 24-h or 25-d exposures, fish were examined using the following techniques. Immediately following the completion of the acute exposure, 24 mL of anaesthetic (50% 2-phenoxyethanol, Sigma, St. Louis, Mo.) in distilled water was injected into each 8-L aquarium to minimize handling stress. Following chronic exposure, fish were carefully netted into a buffered solution of 1:4000 MS 222 (Sigma). Unconciousness was rapid (<20 s) and care was taken to minimize the disturbance of other treatment groups. Each fish was quickly blotted dry and its weight and length recorded. A blood sample was obtained via the caudal vasculature and collected in heparinized capillary tubes. A 4.0-μL aliquot of blood was immediately assayed for hemoglobin concentration (Sigma) and the remaining blood was spun for 5 min in a Clay Adams Autocrit II centrifuge. Following centrifugation, hematocrit and leucocrit were determined and the plasma was separated and stored at −25°C until subsequent assays. The liver and spleen of each fish were removed and weighed. The hepatic and spleen somatic indices (HSI and SSI, respectively) were calculated using the following formula: organ weight × 100·body weight^{-1}. The liver was then placed in individual microcentrifuge tubes, frozen on dry ice, and stored at −25°C. A sample of dorsal white muscle tissue (~0.5 g) was dissected out and stored at −25°C until assayed. Plasma cortisol levels were determined using a commercial radioimmunoassay kit (IncStar Corp., Stillwater, Minn.). Plasma was spectrophotometrically analyzed for cholesterol, lactate, glucose (Sigma) and protein (BioRad Laboratories Ltd., Mississauga, Ont.). Tissue protein, glycogen, and glucose were determined using the methods of Sweeting (1989). All assays were performed on ice in duplicate.

Swimming Performance

The effect of TeCG exposure on maximal swimming performance (U_{crit}) was determined using oval-shaped raceways as described by Farrell et al. (1991). Two or three groups of 10 fish were placed together in the swimming apparatus immediately following TeCG exposure and allowed to acclimate in still water for 3 h before the beginning of the trial. The different groups were identified by an adipose fin clip. Initial water velocity was approximately 1 body length $(BL) \cdot s^{-1}$ based on the average body length of the group. Water velocity was increased by 0.7 $BL \cdot s^{-1}$ every 30 min. A fish was considered exhausted when it rested against the rear grid and did not respond to mechanical stimulation. Time and water velocity at exhaustion were recorded for each fish. The fish was then removed from the apparatus and its body length recorded to calculate U_{crit} using the method of Farrell et al. (1990).

TABLE 1. Effects of an acute 24-h exposure on several parameters in rainbow trout at sublethal TeCG concentrations. SSI, spleen somatic index; Hb, hemoglobin. Values are means ± SE, $n = 9$. Asterisks denote a significant difference from controls at $p < 0.05$.

	TeCG ($mg \cdot L^{-1}$)				
	0	0.1	0.2	0.3	0.4
SSI	0.12 ± 0.02	0.12 ± 0.02	0.11 ± 0.02	0.10 ± 0.03	0.07 ± 0.02*
Lactate ($mg \cdot dL^{-1}$)	25.74 ± 5.04	15.95 ± 3.51	20.90 ± 2.53	24.48 ± 5.44	52.93 ± 11.53*
Hb ($g \cdot dL^{-1}$)	8.84 ± 0.54	9.06 ± 0.38	7.45 ± 0.28	7.16 ± 0.40*	5.89 ± 0.30*
Leucocrit (%)	1.32 ± 0.18	0.72 ± 0.05*	1.07 ± 0.18	1.04 ± 0.14	1.10 ± 0.15
Cortisol ($\mu g \cdot dL^{-1}$)	1.93 ± 0.43	1.29 ± 0.21	1.06 ± 0.16	1.73 ± 0.45	9.76 ± 3.38*
Muscle glycogen ($mg \cdot g^{-1}$)	3.00 ± 1.05	1.78 ± 0.81	1.44 ± 0.81	1.25 ± 1.34	1.00 ± 1.10
Muscle protein ($mg \cdot g^{-1}$)	11.07 ± 0.64	12.02 ± 0.70	11.61 ± 0.33	12.38 ± 0.47	12.38 ± 0.60
Liver glycogen ($mg \cdot g^{-1}$)	95.06 ± 27.88	73.44 ± 14.16	71.33 ± 28.67	11.94 ± 8.12*	0.89 ± 0.69*
Liver protein ($mg \cdot g^{-1}$)	272.8 ± 75.9	184.2 ±14.9	157.3 ± 20.6*	214.8 ± 12.6	155.8 ± 11.2*

Disease Resistance

For the disease challenge, from a total of 50 fish in each treatment, two replicates of 15–20 fish each were randomly selected from each treatment group and exposed to the bacterium *Aeromonas salmonicida*. Of the 50 fish in each treatment, there were no deaths in the acute exposure group and two in the chronic exposure group caused by TeCG exposure alone. *Aeromonas salmonicida* is responsible for the highly infectious fish disease furunculosis. Primary isolates of *A. salmonicida* were cultured from coho salmon (*Oncorhynchus kisutch*) known to have died from furunculosis. A sample of 10 fish from the unexposed stock tank was analyzed before the experiment to ensure that there were no *A. salmonicida* infections present. Bacterial suspensions were prepared in sterile peptone–saline (0.85% NaCl, 0.10% peptone) from 4-d-old *A. salmonicida* cells grown on TSA agar plates at 10°C, and optical density measurements (OD_{540}) were used to provide an estimated concentration of 5×10^5 cells·mL^{-1}. Fish were fin-clipped for identification prior to disease exposure. Using a large sieve, each replicate group was immersed in the aerated bacterial suspension for 15 min and transferred to one of two 1000-L holding tanks. Replicates from within a treatment group were placed in opposite tanks. Holding conditions were as previously described. Dead fish were frozen for later necropsy to verify that furunculosis was the cause of death. Three criteria had to be satisfied for furunculosis to be considered the cause of death: (a) short, Gram-negative, rod-shaped bacteria must be evident in Gram-stained kidney smears, (b) pure cultures of a pigment-producing bacteria resembling the above bacteria must be isolated onto TSA agar from kidney samples, and (c) clinical signs of mortalities must be consistent with those described for *A. salmonicida* infections (Bullock et al. 1983).

LC_{50} Determination

For the LC_{50} determination, eight fish were stocked in each of seven 8-L glass aquaria receiving different nominal TeCG concentrations of 0, 0.10, 0.20, 0.30, 0.40, 0.60, 0.90 $mg \cdot L^{-1}$. The LC_{50} was determined following Sprague (1973). At the conclusion of the trial, all surviving fish were transfered to a 160-L glass aquaria receiving fresh-flowing water for 2 mo to allow continued postexposure observation.

Statistical Analysis

The LC_{50} and 95% confidence limits were calculated according to Finney (1971). With the exception of the disease challenge mortality data, all results were compared with control values using a Student t-test ($p < 0.05$). All percent data were arcsin transformed prior to analysis. Disease challenge mortality curves were compared with controls using the Mantel–Cox (Mantel 1966) and Breslow (Breslow 1970) test statistics ($p < 0.05$).

Results

Biochemical Analysis

Biochemical analyses for the acute trial are summarized in Table 1. Relative spleen size decreased at the highest concentrations in this trial. In addition, the mean hematocrit (data not shown) and hemoglobin values in the acute trial showed a decreasing trend compared with controls. Hemoglobin was significantly lower at the two highest TeCG concentrations. There was little effect on leucocrit levels except for a decrease in the 0.1 $mg \cdot L^{-1}$ treatment group. Liver size was unaffected in any of the treatment groups in the 24-h (acute) trial following TeCG exposure (data not shown) although mean protein values for liver were lower at the highest concentration and liver glycogen was dramatically reduced at the highest concentrations. Plasma cortisol and plasma lactate were greatly elevated at the highest acute TeCG concentration. There was no effect on muscle glycogen and muscle protein in any acute treatment group.

Biochemical analyses for the chronic trial are summarized in Table 2. In contrast with the acute trial, relative spleen size increased at the highest concentrations in this trial and there were no significant differences in hematocrit (data not shown) or hemoglobin levels. Leucocrit levels were significantly elevated relative to control levels in all

TABLE 2. Effects of a 25-d exposure on several parameters in rainbow trout at sublethal TeCG concentrations. SSI, spleen somatic index; Hb, hemoglobin. Values are means ± SE, $n = 9$. Asterisks denote a significant difference from controls at $p < 0.05$.

	TeCG ($mg \cdot L^{-1}$)			
	0	0.02	0.10	0.20
SSI	0.10 ± 0.01	0.14 ± 0.03	0.13 ± 0.02	0.15 ± 0.02*
Lactate ($mg \cdot dL^{-1}$)	24.09 ± 2.07	22.78 ± 3.41	29.40 ± 3.14	28.77 ± 2.59
Hb ($g \cdot dL^{-1}$)	7.66 ± 0.36	7.75 ± 0.28	7.63 ± 0.31	7.50 ± 0.36
Leucocrit (%)	0.50 ± 0.05	0.87 ± 0.07*	0.83 ± 0.07*	1.03 ± 0.08*
Cortisol ($\mu g \cdot dL^{-1}$)	1.36 ± 0.45	0.48 ± 0.15*	0.50 ± 0.18*	0.60 ± 0.11*
Muscle glycogen ($mg \cdot g^{-1}$)	4.10 ± 1.76	6.60 ± 3.02	6.60 ± 1.81	2.50 ± 1.50
Muscle protein ($mg \cdot g^{-1}$)	12.21 ± 0.64	14.64 ± 1.37	13.24 ± 0.41	13.04 ± 0.64
Liver glycogen ($mg \cdot g^{-1}$)	63.10 ± 8.55	41.55 ± 16.86	44.85 ± 16.60	50.95 ± 13.22
Liver protein ($mg \cdot g^{-1}$)	187.73 ± 8.75	201.00 ± 8.38	178.07 ± 22.0	173.49 ± 7.81
Plasma glucose ($mg \cdot dL^{-1}$)	89.14 ± 11.03	81.12 ± 6.07	101.64 ± 8.89	114.85 ± 12.85
Plasma protein ($g \cdot dL^{-1}$)	2.83 ± 0.24	2.69 ± 0.26	3.24 ± 0.26	3.80 ± 0.24*
Plasma cholesterol ($mg \cdot dL^{-1}$)	171.0 ± 13.9	164.2 ± 24.2	166.5 ± 12.6	164.1 ± 12.7

treatment groups. Liver size was unaffected in any of the treatment groups of the 25-d (chronic) trial following TeCG exposure (data not shown). Mean protein and glycogen values for liver were not different from controls. Plasma lactate and glucose were not significantly different from controls, although they exhibited an increasing trend. Plasma protein also exhibited an increasing trend, with a significant elevation at the highest concentration. Plasma cortisol was significantly lower in all treatment groups. There was no effect on muscle glycogen and muscle protein in any chronic treatment group.

Swimming Performance

Swimming performance was affected by acute exposure of trout to TeCG. Mean critical swimming speeds were 32 and 30% less than in control fish in the 0.1 and 0.2 mg $TeCG \cdot L^{-1}$ groups, respectively, following 24 h of exposure (Fig. 1A). Mean critical swimming speeds in treated fish were not significantly different from controls following chronic exposure to TeCG (Fig. 1B).

Disease Resistance

The first mortalities in the disease challenge experiments occurred 10 d following exposure to the bacteria in the acute trial (Fig. 2A) and after the second day in the chronic trial (Fig. 2B). Significant mortalities continued to occur until day 42 and day 15 for the acute and chronic trials, respectively. By day 13, cumulative mortalities in the acute trial were 13% in fish that had been previously exposed to 0.2 mg $TeCG \cdot L^{-1}$. There were no mortalities in any of the other acute trial groups at this time. At day 42, cumulative mortalities in the acute 0.2 $mg \cdot L^{-1}$ group were 89% greater than controls whereas the 0.1 and 0.3 $mg \cdot L^{-1}$ groups were not significantly different from controls. There were no mortalities in the 0.4 $mg \cdot L^{-1}$ group. In the chronic exposure study the highest TeCG treatment group (0.2 $mg \cdot L^{-1}$) had mortalities 108% greater than controls after 15 d. The other treatment groups were not significantly different from the control group.

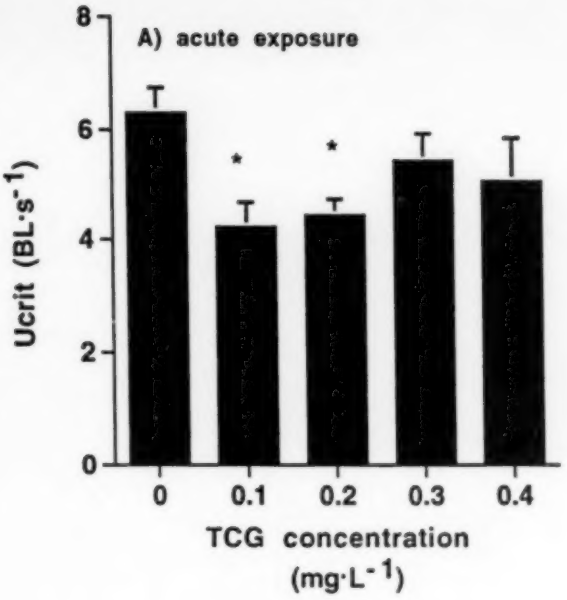
All dead fish displayed typical signs of external pathology associated with *A. salmonicida* infection including areas of focal necrosis in the muscle as a swelling under the skin which in some cases developed into an abscess opening to the surface, appearing as a crateriform lesion. In some fish, necrotic lesions at the base of pelvic and pectoral fins were evident. In the laboratory diagnosis, *A. salmonicida* was isolated from kidney tissue and identification of the bacterium was made according to the criteria outlined previously. In all fish examined, the bacterium *A. salmonicida* was isolated.

LC_{50} Determination

The 96-h LC_{50} for rainbow trout exposed to TeCG was estimated at 0.37 $mg \cdot L^{-1}$ with 95% confidence limits of 0.07. Survivors from this trial were observed for 2 mo post-exposure and no additional mortalities occurred. Even those fish at potentially lethal concentrations that were visibly stressed or incapacitated recovered with no visible lasting effect.

Discussion

The present study was undertaken to examine the effects of sublethal TeCG exposures on juvenile rainbow trout utilizing a suite of indicators that have been shown to be effective in identifying stress in fish (Anderson 1990; Goede and Barton 1990; Schreck 1990; Thomas 1990). Because it has been suggested that greater than 90% of the total toxicity of BKME can be attributed to chlorinated resin acids and guaiacols (Leach and Thakore 1975), some interesting comparisons



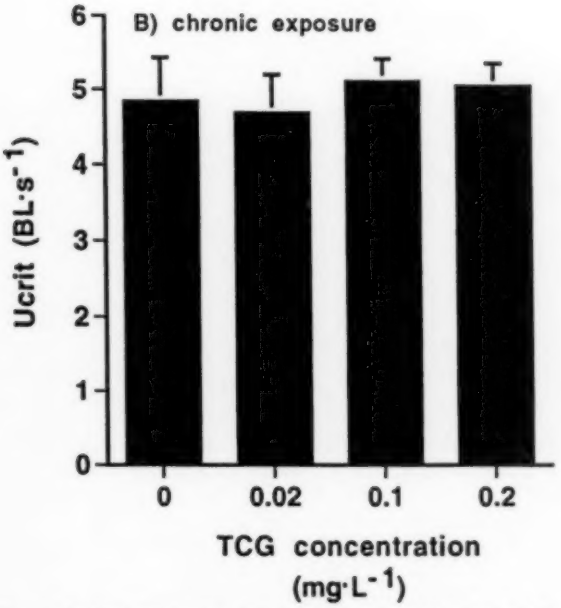
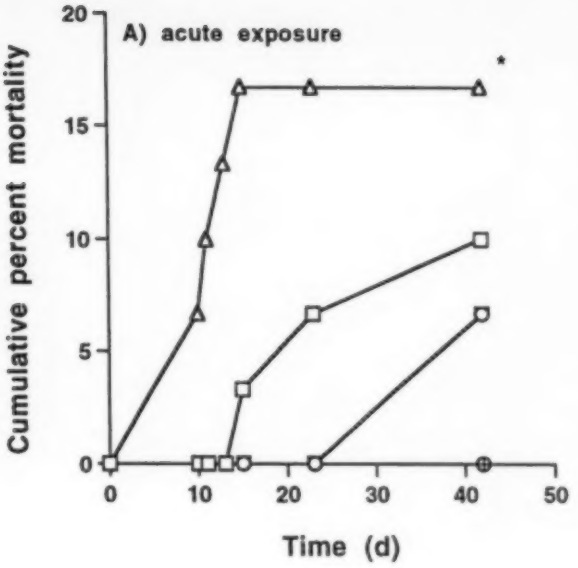


FIG. 1. Critical swimming speeds of juvenile rainbow trout following (A) an acute sublethal 24-h exposure to 0.0, 0.1, 0.2, 0.3 and 0.4 mg TeCG·L^{-1} or (B) a chronic 25-d exposure to 0.0, 0.02, 0.1, and 0.2 mg TeCG·L^{-1}. Values are means ± SE of 10 fish. Asterisks denote a significant difference in critical swimming speeds from control fish at a significance level of $p < 0.05$.



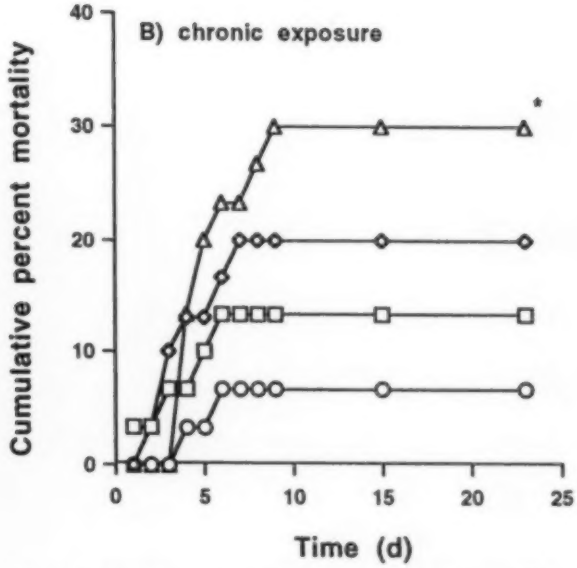


FIG. 2. Cumulative percent mortality of rainbow trout following a challenge to the bacterium *A. salmonicida*. Fish had previously been exposed to (A) an acute sublethal 24-h exposure to 0.0 (□), 0.1 (○), 0.2 (△), 0.3 (▽), and 0.4 (⊕) mg TeCG·L^{-1} or (B) a chronic 25-d exposure to 0.0 (□), 0.02 (◇), 0.1 (○) and 0.2 (△) mg TeCG·L^{-1}. Asterisks denote a significant difference in treatment mortality curve from control fish at a significance level of $p < 0.05$.

can be made between the current study, utilizing a single component of BKME, and studies examining the effects of whole effluent on similar sublethal stress indicators. Our results are consistent with those from other studies and indicate that TeCG, like BKME, can cause a rapid disruption of the homeostasis of the animal followed by a period of adaptation or compensation. It has been suggested that prolonged exposure to a stressor elicits a pattern in fish (McLeay and Brown 1974, 1979; Thomas 1990) similar to that

proposed for mammals by Selye (1950), which consists of three stages: (1) alarm, (2) resistance, and (3) exhaustion. The use of both acute and chronic exposure regimes, as in this study, allows the investigation of these stages.

During the alarm stage, one of the primary responses is hypersecretion of corticosteroid hormones (McLeay and Brown 1974; Schreck 1990) which triggers a host of secondary responses including hyperglycemia, hyperlacticemia, depletion of tissue glycogen, catabolism of tissue proteins, and altered blood levels of protein (Thomas 1990). In addition, a decrease in the number of circulating white blood cells occurs, resulting in a tertiary response characterized by a diminished defense mechanism to infectious agents (Thomas 1990). Many of these responses are reflected in the results in the acute trial of the current study (e.g., an increase in plasma cortisol levels, hyperlacticemia, and a depletion of some tissue glycogen stores), suggesting that these fish were in the alarm stage of stress response.

Following the alarm stage of stress, blood glucose and lactate and liver and muscle glycogen levels return to basal levels during the resistance stage of stress (Thomas 1990). This corresponds to our results from the 25-d exposure to TeCG, in which most of the above parameters were not significantly different from control groups. These results are similar to those of other studies in which salmonids were exposed to BKME. For example, McLeay and Brown (1975) found decreased liver glycogen and increased plasma glucose and lactate levels in coho salmon following 72 h of exposure to BKME whereas following a 30- to 200-d exposure, the magnitude of disturbance to carbohydrate metabolism was much reduced (McLeay and Brown, 1979). Oikari et al. (1988) found no effect of BKME on carbohydrate metabolism in salmonids following long-term exposure.

Oikari et al. (1988) found that BKME can accelerate the breakdown of circulating red blood cells in fish which may be indicated by a decrease in hematocrit and hemoglobin as shown in this study. However, their results followed a 7-wk exposure to simulated BKME whereas the reduction in these parameters in the present study occurred in the acute but not the chronic trial. Relative liver size (HSI) was unaffected by either an acute or chronic exposure to TeCG. In contrast, Oikari et al. (1984) reported a decreased HSI following 30 d of exposure of rainbow trout to BKME; however, Andersson et al. (1987) noted an increase in sculpin LSI after 5–9 months of BKME exposure. Similarly, Oikari et al. (1984) found that leucocrit was significantly elevated in coho salmon following an 11-d exposure to simulated BKME whereas others have shown that leucocrit levels in juvenile coho salmon were depressed after 24 h of exposure to BKME (McLeay and Gordon 1977). In the present study, acute exposure to TeCG significantly decreased leucocrit at the lowest TeCG concentrations whereas chronic exposure led to significant elevations at all concentrations. Relative spleen size (SSI) decreased in the acute trial in the current study and increased in the chronic trial. Fujiya (1961) observed internal hemorrhaging in spleens of fish exposed to pulp mill effluent for 12 and 24 h which may alter spleen weight, although this was not examined in the present study. Stimulation of the release of red blood cells from the spleen into the general circulation may be an early response to stress in fish (Perry and Kinkead 1989) and may also affect spleen weight although an increase in hematocrit was not observed in our study.

Sublethal exposure to effluent may alter the capacity of fish to perform various physiological and behavioural functions. These functions may therefore be useful indicators of stress, particularly as they incorporate several levels of biological organization, are integrative in nature, and provide direct links to the survival of the organism (Schreck 1990). In this study, two performance tests, swimming performance (U_{crit}) and disease resistance, were used to assess the effects of TeCG exposure on rainbow trout. In the acute trials, swimming performance was reduced in fish exposed to the lower concentrations of TeCG, but not at higher concentrations. Moreover, there was no inhibitory effect of TeCG on swimming performance in the chronic trials at any concentration. Howard (1975) also reported an impaired critical swimming speed in juvenile coho salmon following an acute exposure to BKME whereas McLeay and Brown (1979) found no impairment in swimming capacity following a 90-d exposure of juvenile coho salmon to BKME. It has been suggested that the decrease in swimming performance during acute exposure of fish to BKME could be due to a reduction in respiratory efficiency (Davis 1973). This is supported by the finding that several components of BKME, including TeCG, are uncouplers of mitochondrial oxidative phosphorylation (Lundberg et al. 1980). The reason for the similar values for U_{crit} in the control and highest TeCG concentration groups of the acute trial is unclear; however, swimming performance did not appear to be affected by chronic exposure to TeCG, possibly due to adaptive or compensatory mechanisms.

Stress is known to suppress the immune system in fish (Maule et al. 1989) and direct assessment of immune system competence can be used as a powerful stress indicator (Anderson 1990). The results of the disease resistance challenge experiments indicate that both acute and chronic exposures of fish to sublethal TeCG concentrations result in a reduced ability to resist a pathogen. Generally, fish exposed to TeCG died from furunculosis significantly earlier and at greater rates than did unexposed fish. However, there were reduced mortalities in fish that were exposed to TeCG at the highest concentration in the acute trial. It is possible that residual TeCG in the fish tissues acted as an antibacterial agent, possibly protecting the fish from the initial infection. Other compounds such as the chlorinated phenols are also uncouplers of oxidative phosphorylation and have been used as antibacterial agents (Kennedy 1990). Lowered disease resistance, such as that indicated by the results of the present study, may arise from increases in plasma cortisol levels from contaminant-induced stress. Elevated cortisol has been documented to result in repression of several defence mechanisms (Anderson et al. 1982). However, the effect(s) of cortisol on the immune system in the present study is unclear because the chronically exposed fish had lower levels of plasma cortisol than did controls, but were still more susceptible to *A. salmonicida*. This result may be a reflection of changes in either the number of cortisol receptors or cortisol clearance rates from the plasma, both of which have also been shown to change in fish under stress conditions (Brown et al. 1990; Maule and Schreck 1991). Additionally, initial plasma cortisol levels in the chronic trial may have been elevated, producing a long-term effect on the immune system.

This study has identified a suite of stress indicators (plasma cortisol, glucose, lactate, liver glycogen, swimming

performance, and disease resistance) that may be useful in monitoring stress in fish exposed to TeCG. These indicators have shown that rainbow trout exhibit classic primary (hormonal), secondary (biochemical), and even tertiary (reductions in swimming performance and disease resistance) stress responses after acute exposure to TeCG, which may ultimately affect survival. The results further indicate that compensation may occur if fish are chronically exposed to contaminants. This compensation or resistance could also explain the apparent recovery of the fish following the LC_{50} determination, although this was not comprehensively examined. The 96-h LC_{50} of TeCG would classify it as very acutely toxic according to Sprague (1973). This classification, along with the consistency of our results with studies using whole effluent, supports Leach and Thakore's (1975) suggestion that chloroguaiacols are one of the major contributors to BKME fish toxicity. If this is indeed the case, then developing an effective detoxification treatment for the chloroguaiacols would be useful in reducing the effect of BKME on fish populations.

Acknowledgements

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Influence of Sustained Exercise at Two Ration Levels on Growth and Thyroid Function of All-Female Chinook Salmon (*Oncorhynchus tshawytscha*) in Seawater

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Kiessling, A., D.A. Higgs, B.S. Dosanjh, and J.G. Eales. 1994. Influence of sustained exercise at two ration levels on growth and thyroid function of all-female chinook salmon (*Oncorhynchus tshawytscha*) in seawater. Can. J. Fish. Aquat. Sci. 51: 1975–1984.

Duplicate groups of seawater-adapted 70-g all-female chinook salmon (*Oncorhynchus tshawytscha*) were held in flowing seawater (24–29‰; 8–12°C) corresponding to swimming speeds (SS) of 0.5, 1.0, or 1.5 body lengths (bl)/s for 212 d. Fish were fed either a maximum satiating ration of 75% of maximum ration at each SS. Fish grew four- to fivefold during the study, but neither mean body weight, fork length, specific growth rate, condition factor, nor muscle morphometry was influenced by average SS. However, the SS of individually marked fish were negatively correlated with specific growth rate. At both rations, higher SS increased the internal tissue-carcass ratio, hepatosomatic index, and total body protein. More food was ingested at higher SS resulting in reduced feed efficiency. SS did not alter the plasma L-thyroxine (T_4) concentration, but increased the plasma 3,5,3′-triiodo-L-thyronine (T_3) concentration and T_3/T_4 ratio. In part, this may reflect the greater food intake at higher SS. The reduced ration depressed all aspects of growth and at 212 d tended to depress both plasma T_4 and T_3, but did not alter feed efficiency. We conclude that exercise over the range of 0.5–1.5 bl/s does not enhance growth, but reduces feed efficiency in seawater-adapted chinook salmon.

Des groupes préparés en double, composés entièrement de femelles de saumon quinnat (*Oncorhynchus tshawytscha*) adaptées à l'eau de mer 70 g, ont été conservés dans de l'eau de mer en circulation (24–29 ‰; 8–12 °C) à une vitesse correspondant à des vitesses de nages (VN) de 0,5, 1,0 ou 1,5 longueurs corporelles (lc)/s pendant 212 jours. Les poissons ont reçu une ration maximale de satiété ou 75 % de la ration maximum à chaque VN. La croissance des poissons correspondait à un facteur de 4–5 pendant l'étude, mais leur poids corporel moyen, leur longueur à la fourche, leur taux de croissance spécifique et leur facteur de condition pour la morphométrie musculaire n'ont été influencés par des valeurs moyennes de VN. Toutefois, les VN d'individus marqués présentaient une corrélation négative avec des taux de croissance spécifiques. Aux deux rations, les valeurs supérieures de VN augmentaient le rapport interne tissu/carcasse, l'indice hépatosomatique et les protéines corporelles totales. Plus d'aliments étaient ingérés aux valeurs supérieures de VN, ce qui a entraîné une efficacité réduite de l'alimentation. Les VN n'ont pas modifié les valeurs de concentration de la L-thyroxine (T_4) du plasma, mais elles ont augmenté la concentration de la 3,5,3′-triiodo-L-thyronine (T_3) du plasma, ainsi que le rapport T_3/T_4. Ceci peut être dû en partie à l'ingestion de grandes quantités d'aliments aux valeurs supérieures de VN. La ration réduite correspondait à une diminution de tous les aspects de la croissance et, après 200 jours, elle tendait à abaisser les valeurs de T_4 et de T_3 du plasma, mais ceci n'a pas modifié l'efficacité de l'alimentation. Nous concluons donc que l'exercice à une plage supérieure à 0,5–1,5 lc/s n'augmente pas la croissance, mais réduit l'efficacité de l'alimentation chez les saumons quinnats adaptés à l'eau de mer.

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The benefits of continuous swimming (sustained exercise) on salmonid growth have been evaluated in several studies (reviewed by Davison 1989). Sustained aerobic exercise has been reported to increase body growth, muscle fibre hypertrophy, and feed efficiency and to improve the final culinary quality (Greer-Walker 1971; Davison and Goldspink 1977; Greer-Walker and Emerson 1978; Besner 1980; Johnston and Moon 1980; Kuiper 1982; Nahhas et al. 1982; Leon 1986; East and Magnan 1987; Houlihan and Laurent 1987; Totland et al. 1987; Christiansen et al. 1989; Christiansen and Jobling 1990; Farrell et al. 1990). Also, physical exercise may shift substrate utilization from protein to lipid (Christiansen et al. 1989) and increase protein synthesis and net protein deposition in white muscle (Houlihan and Laurent 1987). Underlying these changes may be alterations

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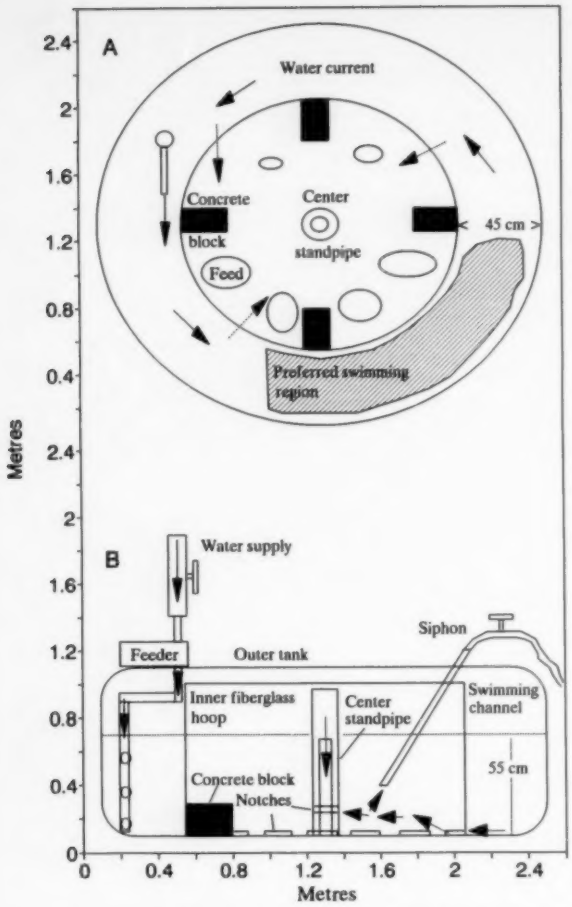

FIG. 1. Schematic drawing of one of the experimental tanks. (A) Top view; (B) side view. Arrows signify the direction of water flow.

in growth-regulating endocrine systems (Higgs and Eales 1971; Barret 1988).

In previous studies of the effects of exercise, the control salmonids were held in essentially static water with no defined pattern of flow. This was done to reduce locomotory activity and energetic demands. However, in such static systems, there may be considerable behavioural activity associated with aggression, resulting in stressful biting and fin-nipping and elevated plasma levels of cortisol. Elevated cortisol levels are known to suppress growth (Pickering 1990) and feed efficiency (Vijayan and Leatherland 1989; Van der Boon et al. 1991). In contrast, when salmonids are held in a current, they school and swim less randomly (Totland et al. 1987; Christiansen et al. 1989; Christiansen and Jobling 1990; Boisclair and Tang 1993). Moreover, they exhibit less aggression as revealed by reduced fin-nipping and bite marks (Christiansen et al. 1989; Christiansen and Jobling 1990) and have lower levels of plasma cortisol indicating reduced stress (Woodward and Smith 1985; Boesgaard et al. 1993a (Atlantic salmon (*Salmo salar*)), 1993b; Postlethwaite and McDonald 1993 (rainbow trout (*Oncorhynchus mykiss*)). Thus, the reported benefits in growth resulting from exercise may stem not from the exercise per se, but from the diminished energy demands of stressful aggressive interactions. On this basis, we determined the effects of swimming speed on growth and related parameters under conditions in which the above potentially confounding behavioral effects could be eliminated.

In an attempt to determine the combination of physical activity and ration level that would enable more cost-efficient production of farmed salmon, we have investigated the effects of three levels of sustained submaximal aerobic exercise on the growth of seawater-adapted chinook salmon (*Oncorhynchus tshawytscha*). Control fish were kept in the slowest possible current in which they maintained position and schooled uniformly. Increased levels of physical activity were created by doubling and tripling this swimming speed. By this design, we aimed at minimizing the potentially confounding effects of random swimming, social aggressive interactions, and territorial behaviour around the feeder. Plasma levels of thyroid hormones were measured also as indices of thyroid function, which may change in response to nutrient intake (Eales 1988; Eales et al. 1992), growth (Higgs et al. 1982), and alteration in current velocity (Higgs and Eales 1971).

Material and Methods

Fish and Experimental Conditions

In December 1991, 660 all-female (Solar et al. 1987) seawater-adapted, hatchery-raised (Sea Spring Salmon Farm Ltd., Chemainus, B.C. Canada) 1-yr-old Qualicum chinook salmon were selected for uniform size (mean weight = 75 g ± 20, range) from a total of 1200 fish. Of these, 300 fish were marked individually with PIT tags (BioSonics, Seattle, Wash., U.S.A.). Both tagged and untagged fish were divided equally into 12 groups by placing them, five at a time, into identical 4-m^3 circular outdoor fibreglass tanks (each group had 25 tagged and 30 untagged fish).

Each tank was fitted with an inner fibreglass hoop (diameter 1.5 m) creating a circular swimming channel 45 cm wide and 55 cm deep (Fig. 1). Half of each tank was covered with plastic netting and the other half with black nylon cloth to allow the fish to choose freely between illuminated and shaded regions. Seawater was pumped into each tank through a vertically placed pipe equipped with three 30-cm-long horizontally oriented pipes, functioning as inlet jets. The diameter of the latter was varied to modify water velocity, so that each tank was provided with approximately the same overall water flow rate. The system was arranged as a flow-through system with no recirculation of the water. The water flow carried uneaten food and faeces from the outer channel to the inner compartment through several notches located on the bottom of the inner fibreglass hoop (Fig. 1). Notches were placed also on the central outer standpipe 15 cm from the tank bottom. This ensured that uneaten pellets settled in the inner compartment. Uneaten pellets were counted every second day to determine food eaten and then removed by siphon. Supplemental water aeration was provided by millepore tubing connected to compressed air. Water flow rate, temperatures, salinities, and dissolved oxygen concentrations during the experiment ranged from 25 to 40 L/min, from 8.2 to 12.3°C, from 24 to 29‰, and from 11 to 14 ppm, respectively.

Experimental Design

A two-by-three factorial design was employed with two ration levels (maximum ration = RL100, 75% of maximum ration = RL75) and three water velocities (0.5, 1.0, and 1.5 body lengths (bl)/s. Duplicate groups of fish were assigned to each of the six treatments.

The daily rations ingested were estimated by subtracting the uneaten food (number of uneaten pellets·mean pellet weight (grams) before water exposure) from the daily amount of food dispensed. Following water exposure for 2 d the extruded pellet had a changed color and size indicating leaching and uptake of water. However, disintegration of the pellet structure (crumbling) was not evident before 3–6 d in the water, making the identification of single pellets exposed to water for shorter periods uncomplicated. For RL100 fish, food waste represented 5–10% of the prescribed ration. The RL75 fish were given 75% of the ration given to RL100 fish with an equal swim speed (SS). This portion was adjusted for differences in biomass. Ration levels were increased daily, taking increases in fish biomass, changes in water temperature, and feed wastage into account. Because different amounts of food were offered to fish swimming at different speeds and because feed waste differed between rations, the true reduced ration ($RL75_T$) was calculated for fish held at each SS as follows: (RL75 (given feed − waste)·RL100^{-1} (given feed − waste))·100, with RL expressed as feed given per kilogram of fish. The actual rations ingested by the fish denoted RL75 were not affected by SS ($p > 0.7$). The mean values of $RL75_T$ for SS 0.5, 1.0, and 1.5 were 77.8 ± 0.9 SE, 79.4 ± 0.9, and 78.5 ± 0.6, respectively.

All fish, regardless of treatment, were fed 4- to 6-mm Biodry 2500 pellets (Bioproducts Inc., Warrenton, Oreg., U.S.A.). The mean levels (wet weight basis) of proximate constituents and gross energy of different batches of this diet were as follows: % protein, 46.1 ± 0.8 (SE); % lipid, 17.9 ± 1.5; % ash, 11.2 ± 0.5; % nitrogen-free extract, 13.4 ± 0.9; % dry matter, 88.6 ± 1.0; energy, 4836 ± 88, kcal/kg. The pellet density was such that pellets were carried around the tank, irrespective of water velocity, before reaching the bottom and being swept into the inner compartment. The prescribed rations were dispensed continuously into each tank by belt feeders (Zeigler Bros. Inc., Gardners, Pa., U.S.A.) from 08.00 to 10.00 and from 15:00 to 17:00. All groups were fed half of their daily ration 24–26 h before weighing and sampling.

Water velocities in different parts of the circular swimming channel were measured in each tank every second day using a flowmeter (Braystoke BFM002, Valeport Marine Scientific, Devon, U.K.). No differences were found between the top and bottom of the water column. However, relative to the centre of the channel, a 5–10% decrease in water velocity was noted close to the inner and outer walls. A region of turbulence and higher water velocity was found from the point of water entry to 1 m downstream. Otherwise, good laminar flow was noted based on movement of air bubbles distributed throughout the water column. Confinement of fish to a specific region of the channel was unnecessary, as fish situated themselves downstream of the feeder in a region of consistent laminar flow (Fig. 1).

Fish Weighing, Sampling, and Analysis

Before weighing and sampling, the fish were anaesthetized according to the protocol of Kreiberg and Powell (1991), using Marinil® (0.25 ppm) and MS222® (60 ppm).

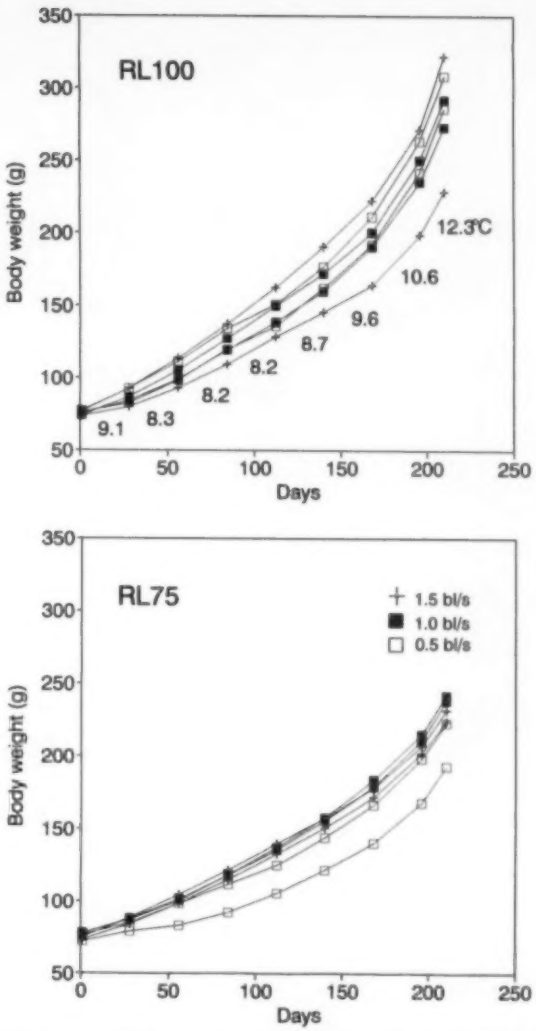

FIG. 2. Temporal increases in body weight in duplicate groups of seawater-adapted chinook salmon exposed to different levels of sustained exercise (expressed as body lengths per second) and ration levels (RL, expressed in percent of satiation ration). Mean water temperatures for each sampling interval are given for the RL100 fish.

All fish in each group were weighed individually (body weight, Bwt) to the nearest 0.1 g and measured (fork length, FL) to the nearest 1 mm every 28 d (last interval 16 d, Fig. 2) throughout the 212-d study. Plasma samples and samples for chemical and body conformation measurements were taken from untagged fish as follows: two per tank (total of 24) on day 0; six per tank on day 84. On day 212, samples were taken from 12 tagged fish per tank.

Blood samples were collected in heparinized vacutainers from the caudal vessels. Subsequently, the samples were placed onto ice, centrifuged at 4500*g* for 5 min, frozen with either dry ice or liquid nitrogen, and stored at −80°C. Fish and muscle samples for proximate analysis were immediately frozen on dry ice, vacuum packed, and stored at −20°C.

TABLE 1. Growth and body condition of chinook salmon. Bwt, body weight; FL, fork length; SGR, specific growth rate; CP, carcass percent; *K*, condition factor; HSI, hepatosomatic index. Values are the mean of duplicate groups ± SE. Two tanks showed anomolous growth during the study, due to freshwater contamination. Values for the unaffected replicate are given within parentheses. A *p*-value less than 0.05*, 0.01**, and 0.001*** signifies the level of significance. Statistics were performed using multifactorial analysis for treatments SS (swim speed), RL (ration level), and SS·RL interaction.

		RL100			RL75			*p*		
	Days	SS 0.5	SS 1.0	SS 1.5	SS 0.5	SS 1.0	SS 1.5	SS	RL	SS·RL
Bwt (g)	0	76 ± 2	74 ± 2	75 ± 2	74 ± 2	76 ± 2	74 ± 2	ns	ns	ns
	84	127 ± 4	123 ± 4	124 ± 4 (138)	102 ± 8 (112)	118 ± 4	118 ± 4	ns	***	ns
	212	297 ± 6	282 ± 6	275 ± 7 (322)	208 ± 5 (223)	240 ± 6	228 ± 6	ns	***	ns
FL (mm)	0	188 ± 2	186 ± 1	187 ± 1 (188)	186 ± 1 (188)	188 ± 1	186 ± 1	ns	ns	ns
	84	213 ± 1	211 ± 2	212 ± 2 (219)	202 ± 2 (208)	211 ± 2	209 ± 1	ns	***	ns
	212	277 ± 2	272 ± 2	269 ± 2 (283)	252 ± 2 (257)	264 ± 2	258 ± 2	ns	***	*
SGR	0–84	0.67 ± 0.02	0.65 ± 0.02	0.65 ± 0.02 (0.73)	0.40 ± 0.04 (0.46)	0.57 ± 0.02	0.60 ± 0.03	ns	***	ns
	84–212	0.70 ± 0.02	0.69 ± 0.02	0.63 ± 0.02 (0.67)	0.60 ± 0.02 (0.57)	0.58 ± 0.01	0.55 ± 0.02	ns	***	*
	168–212	0.90 ± 0.09	0.87 ± 0.05	0.89 ± 0.03 (1.01)	0.78 ± 0.10 (0.72)	0.73 ± 0.03	0.75 ± 0.03	ns	***	ns
CP (%)	0	85.5 ± 1.6	85.5 ± 1.6	85.5 ± 1.6	85.5 ± 1.6	85.5 ± 1.6	85.5 ± 1.6			
	84	86.9 ± 0.3	85.4 ± 0.5	85.2 ± 0.4 (84.9)	86.4 ± 0.2 (87.1)	85.2 ± 0.94	85.5 ± 0.3	*	ns	ns
	212	87.2 ± 0.3	87.4 ± 0.4	86.3 ± 0.3 (86.2)	87.7 ± 0.1 (88.6)	88.2 ± 0.2	87.5 ± 0.3	*	ns	ns
K	0	1.15 ± 0.01	1.14 ± 0.01	1.14 ± 0.01 (1.14)	1.14 ± 0.01 (1.14)	1.14 ± 0.01	1.14 ± 0.01	ns	ns	ns
	84	1.28 ± 0.01	1.29 ± 0.01	1.28 ± 0.01 (1.30)	1.18 ± 0.01 (1.21)	1.24 ± 0.01	1.26 ± 0.01	ns	***[a]	ns
	212	1.40 ± 0.02	1.40 ± 0.02	1.40 ± 0.02 (1.40)	1.30 ± 0.02 (1.30)	1.30 ± 0.02	1.30 ± 0.02	ns	***[a]	ns
HSI (%)	84	1.2 ± 0.0	1.4 ± 0.0	1.7 ± 0.1 (1.5)	1.2 ± 0.0 (1.1)	1.3 ± 0.0	1.4 ± 0.0	***	**	**
	212	1.2 ± 0.1	1.1 ± 0.0	1.2 ± 0.0 (1.2)	1.0 ± 0.0 (1.1)	1.0 ± 0.0	1.2 ± 0.1	**	*	**

[a]This effect was negated by adding body weight as a covariate to the model.

Body conformation measurements were made anterior to the base of the first ray of the dorsal fin according to Kiessling et al. (1991) for rainbow trout. Muscle width (MW) was measured as total width at the lateral line, muscle height (MH) was determined between the spinal column and the most dorsal tip of the epaxial white muscle, and muscle fibre length (MFL) was measured at the lateral line as the length of the myotome between two myosepta.

Whole-body and muscle (fillet) proximate compositions were determined according to Higgs et al. (1979).

Plasma thyroxine (T_4) and 3,5,3′-triiodo-L-thyronine (T_3) titres were determined by radioimmunoassay (Omeljaniuk et al. 1984).

Growth Variables

The following variables were calculated: specific growth rate (SGR; (%/d)) = $(\ln_{\text{Bwt end}} - \ln_{\text{Bwt start}} \cdot 28^{-1}) \cdot 100$, condition factor ($K$) = (Bwt (g)·FL^{-3}(cm))·100, feed efficiency (FE) = Bwt gain (g)·food intake^{-1} (dry weight) per 28-d weighing period, carcass percent (CP) = eviscerated Bwt (carcass (g))·100·Bwt^{-1}, hepatosomatic index (HSI) = liver weight (g)·100·Bwt^{-1}, and swim speed (SS) of individual tagged fish = $((\ln_{\text{FLstart}} - \ln_{\text{FLend}})$·days of evaluated growth period^{-1})·mean water current for the same period^{-1}.

Statistical Analyses

Statistical analyses were conducted using the general linear model procedure (GLM) of the Statistical Analysis System (SAS) software package (SAS 1989). The *F*-test was used to test for significant differences between treatment groups and the *t*-test for paired comparisons between muscle sample sites and types within individual fish. Data were analyzed by factorial models including the main factors (SS and RL, interactions). The effects of replicates (tanks) was included in a separate model as a nested factor. The procedure for repeated samples was used when testing for the same main factors on Bwt, FL, SGR, and FE over time. Simple regression was used to evaluate the relationships between individual fish (tagged fish) SS and SGR. In addition, covariate models were performed in cases where a dependence between body constituents and Bwt and between body conformation factors and FL could be expected.

Between days 35 and 37, one of the duplicate tanks for each of RL100/1.5 and RL75/0.5 was contaminated with freshwater from a burst pipe. This was believed to have affected growth (Fig. 2), and statistics were calculated with and without these putative anomolous duplicates (see values in parentheses in Tables 1 and 2). Tank (replicate) did not influence body composition variables and thyroid hormone levels (Tables 3 and 4).

Results

Mortality and Behaviour

Mortality was not related to either SS or RL and constituted a total of 24 fish evenly distributed between tanks. The fish that died never fed (runts). Consequently, they lost weight and death occurred within 2–4 mo depending on SS (sooner for fish at higher SS). Fish always swam upstream (Fig. 1) except for the runts. Several days before death, these fish sought slower water velocities and back eddies close to the side of the tank, and a few hours before death, they were swept downstream with the current.

TABLE 2. Feed ration (RL), feed waste (Waste), and feed efficiency (FE). Values are the mean of duplicate groups ± SE. For further details, see Table 1.

		RL100			RL75			*p*		
	Days	SS 0.5	SS 1.0	SS 1.5	SS 0.5	SS 1.0	SS 1.5	SS	RL	SS·RL
RL (%)[a]	1–84	0.95 ± 0.10	1.04 ± 0.08	1.21 ± 0.12 (1.10)	0.70 ± 0.06 (0.68)	0.78 ± 0.07	0.83 ± 0.04			
	84–212	0.87 ± 0.08	0.94 ± 0.09	1.02 ± 0.09 (1.02)	0.66 ± 0.06 (0.66)	0.70 ± 0.07	0.77 ± 0.08			
	1–212	0.90 ± 0.06	0.98 ± 0.06	1.09 ± 0.08 (1.05)	0.67 ± 0.04 (0.67)	0.73 ± 0.05	0.79 ± 0.05			
Waste (%)[b]	1–84	8.3 ± 2.2	9.4 ± 1.4	11.9 ± 3.6 (3.8)	8.3 ± 1.7 (4.7)	4.2 ± 2.5	3.6 ± 2.4	ns	***	ns
	84–212	6.4 ± 1.7	7.0 ± 1.7	7.7 ± 1.5 (3.8)	1.0 ± 0.3 (0.8)	0.3 ± 0.1	0.4 ± 0.1	ns	***	ns
	168–212[d]	3.2 ± 0.9	3.9 ± 1.5	4.9 ± 1.2 (2.4)	0.4 ± 0.1 (0.5)	0.3 ± 0.1	0.5 ± 0.2	ns	***	ns
FE[c]	1–84	0.89 ± 0.07	0.74 ± 0.07	0.58 ± 0.05 (0.75)	0.66 ± 0.08 (0.90)	0.86 ± 0.03	0.85 ± 0.02	***	ns	ns
	84–212	1.02 ± 0.05	0.90 ± 0.04	0.77 ± 0.05 (0.84)	1.07 ± 0.06 (1.05)	0.94 ± 0.04	0.82 ± 0.02	***	ns	ns
	168–212[d]	1.08 ± 0.03	0.96 ± 0.02	0.82 ± 0.05 (0.88)	1.12 ± 0.04 (1.08)	1.00 ± 0.04	0.85 ± 0.02	***	ns	ns

[a]RL = (daily ration offered·body weight^{-1})·100 (both in wet weight).
[b]Waste = (feed waste·total feed offered^{-1})·100 (both in wet weight).
[c]FE = biomass gain (in wet weight)·eaten feed^{-1} (in dry weight).
[d]Period with most rapid growth (see Fig. 2).

TABLE 3. Proximate composition and energy content of whole-body and muscle (skinned fillet) of fish in relation to treatment (RL to SS) at day 212. Values for day 0 also given. Values (% of tissue wet weight) are expressed as the mean of duplicate groups ± SE. COH_c, calculated carbohydrate content; HG_c, calculated energy content. For further details, see Table 1.

		RL100			RL75			*p*		
		SS 0.5	SS 1.0	SS 1.5	SS 0.5	SS 1.0	SS 1.5	SS	RL	SS·RL
Whole										
Day	0	212								
Protein	17.0 ± 0.3	16.4 ± 0.1	16.9± 0.2	17.0 ± 0.1	16.8 ± 0.2	16.5 ± 0.1	17.1 ± 0.2	***	ns	**
Fat	4.9 ± 0.5	9.0 ± 0.6	8.8 ± 0.5	8.0 ± 0.7	6.1 ± 0.4	6.8 ± 0.5	5.9 ± 0.4	ns	***[c]	ns
Ash	2.16 ± 0.07	1.69 ± 0.08	1.87 ± 0.06	1.84 ± 0.06	1.91 ± 0.03	1.76 ± 0.05	1.87 ± 0.08	ns	ns	ns
COH_c[a]	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.6 ± 0.2	0.7 ± 0.2	1.1 ± 0.3	0.5 ± 0.1	ns	ns	ns
DM	24.6 ± 0.6	27.5 ± 0.4	28.0 ± 0.5	27.3 ± 0.6	25.5 ± 0.5	26.1 ± 0.6	25.4 ± 0.5	ns	***[c]	ns
HG (cal/g)	1414 ± 62	1755 ± 49	1720 ± 42	1732 ± 66	1575 ± 68	1641 ± 58	1515 ± 45	ns	***[c]	ns
HG_c[b]	1446 ± 62	1793 ± 48	1804 ± 47	1733 ± 61	1557 ± 38	1616 ± 46	1548 ± 40	ns	***[c]	ns
Fillet										
Protein	18.5 ± 0.2	19.8 ± 0.2	19.6 ± 0.2	19.7 ± 0.2	19.8 ± 0.2	19.9 ± 0.4	19.6 ± 0.1	ns	ns	ns
Fat	2.4 ± 0.2	5.7 ± 0.8	5.8 ± 0.3	5.4 ± 0.8	2.9 ± 0.4	3.2 ± 0.4	4.2 ± 0.7	ns	***[c]	ns
Ash	1.73 ± 0.02	1.81 ± 0.04	1.75 ± 0.05	1.85 ± 0.04	1.68 ± 0.04	1.79 ± 0.05	1.81 ± 0.10	ns	ns	ns
COH_c[a]	0.4 ± 0.1	0.3 ± 0.2	0.4 ± 0.2	0.8 ± 0.6	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	ns	ns	ns
DM	23.5 ± 0.1	27.6 ± 0.6	27.4 ± 0.4	27.7 ± 0.7	24.8 ± 0.6	25.2 ± 0.6	25.9 ± 0.7	ns	***[c]	ns

[a]Calculated as 100 − (%moisture + protein + fat + ash).
[b]Calculated using caloric equivalents for protein, 5.65 kcal/g; lipid, 9.45 kcal/g; carbohydrate, 4.1 kcal.g.
[c]This effect was negated by adding body weight as a covariate to the model.

TABLE 4. Plasma levels of thyroid hormones (T_4 and T_3) and T_3/T_4 ratios for seawater-adapted chinook salmon subjected to different swim speeds (SS) and fed one of two ration levels (RL). Values for either T_4 or T_3, from the same sampling time followed by the same italic letter, do not differ significantly ($p > 0.05$).

Sampling time (d)	Current (bl/s)	RL (%)	T_4 (ng/mL)	T_3 (ng/mL)	T_3/T_4
0	0	100	4.04 ± 0.76	1.20 ± 0.22	0.40 ± 0.11
84	0.5	100	3.21 ± 0.33*a*	1.71 ± 0.28*a*	0.55 ± 0.06*a*
	1.0	100	2.83 ± 0.38*a*	1.95 ± 0.33*a*	0.76 ± 0.06*b*
	1.5	100	2.62 ± 0.37*a*	1.89 ± 0.27*a*	0.73 ± 0.09*b*
	0.5	75	2.77 ± 0.32*a*	1.64 ± 0.33*a*	0.67 ± 0.19*ab*
	1	75	2.68 ± 0.28*a*	2.20 ± 0.32*a*	0.93 ± 0.21*ab*
	1.5	75	2.86 ± 0.39*a*	1.90 ± 0.28*a*	0.64 ± 0.06*ab*
212	0.5	100	4.83 ± 0.40*a*	2.21 ± 0.16*a*	0.47 ± 0.05*a*
	1	100	5.23 ± 0.45*a*	2.37 ± 0.14*a*	0.48 ± 0.05*a*
	1.5	100	5.01 ± 0.32*a*	2.76 ± 0.24*b*	0.61 ± 0.09*ab*
	0.5	75	3.67 ± 0.31*bc*	1.38 ± 0.16*c*	0.41 ± 0.04*a*
	1	75	3.43 ± 0.39*b*	1.72 ± 0.18*c*	0.52 ± 0.07*ab*
	1.5	75	4.67 ± 0.69*ac*	2.45 ± 0.26*ab*	0.65 ± 0.13*b*

Whole-Body and Muscle Growth

The growth (Bwt, FL, and SGR) of PIT-tagged fish was identical to that of nontagged fish ($p > 0.7$). The temporal changes in Bwt are shown in Fig. 2, and growth variables are provided in Table 1.

Ration level, but not SS, influenced growth (Bwt, FL, and SGR, $p < 0.0001$) with no differences between duplicate tanks when the two anomolous (freshwater-contaminated) tanks were excluded. This was true both during the initial period with slow growth (low temperature, 0–84 d) and during the later period with higher growth (rising temperatures, 168–212 d) (Fig. 2). However, when individual SS of fish within a group were substituted for the average SS (0.5–1.5) in a regression model (instead of a main effect), a negative correlation was found between SS and SGR ($p < 0.01$, $r = -0.42$). No consistent interaction was found between SS and RL on these growth variables (Table 1).

Both the condition factor (K) and fish musculature increased with growth. Muscle conformation variables between day 0 and day 212 increased as follows: MW, 11.3–11.8 ± 0.2% SE; MH, 8.2–9.9 ± 0.04%; MFL, 0.81–0.94 ± 0.01% of FL. No effect of treatment (SS, $p > 0.7$ or RL, $p > 0.3$) was noted on these variables after 212 d if Bwt or FL were added as covariates in the statistical model. Body conformation variables (Bwt, FL, MW, MH, and MFL) were all highly correlated with each other: FL to MW ($r^2 = 0.88$), MH ($r^2 = 0.75$), or MFL ($r^2 = 0.66$) and MW to MH ($r^2 = 0.88$) and the natural logarithm for Bwt to the natural logarithm for FL ($r^2 = 0.97$). The regression coefficients (slopes) of the linear regressions between the above pairs of variables were uninfluenced by SS.

Both SS and RL significantly increased HSI and there was a significant interaction between these variables (Table 1).

Feed Waste and Feed Efficiency

Two apparent opposing factors affected the amount of feed waste. Fish maintained on RL75 were offered less feed compared with those kept on RL100 which resulted in a marked reduction in the amount of waste ($p < 0.0001$). A decrease in waste was also found as growth rate increased in accordance with elevated water temperatures ($p < 0001$; Table 2; Fig. 2). This occurred despite elevated absolute amounts of dispensed feed.

Increased SS depressed ($p < 0.0001$) feed efficiency whereas RL had no effect (Table 2). The effect of SS was less marked early in the study when low water temperatures and short day lengths prevailed (Fig. 2; Table 2). However, as overall fish growth rates increased, the negative effect of SS on FE became more evident. From day 84 onwards, decreases in FE of 11 and 14% were found when comparisons were made between fish exposed to 0.5 versus 1.0 and 1.0 versus 1.5 bl/s, respectively.

Whole-Body and Muscle Proximate Composition

Whole-body protein was increased by SS (Table 3; $p < 0.0008$; $r^2 = 0.3$) independent of ration level. Whole-body and muscle lipid, dry matter, and caloric content were elevated by increased ration ($p < 0.0001$). However, these variables also correlated with Bwt (r^2 = whole body ~0.4 and muscle ~0.5, $p < 0.0001$; Table 3). Bwt was therefore added as a covariate to the statistical model. The addition of Bwt in covariance model negated the effect of ration level on the preceding variables, indicating an effect of attained Bwt rather than a direct cause and effect of RL.

Calculated energy contents (based on caloric equivalents for energy yielding nutrients) of feed (data not shown) and whole fish (Table 3) showed excellent agreement with those determined by bomb calorimetry ($r^2 = 0.99$, $p < 0.0001$).

Thyroid Function

SS and RL had no influence at 84 d on plasma levels of T_4 and T_3, but T_3/T_4 was increased by highest SS for RL100 (Table 4). By 212 d, T_3 increased with both SS ($r^2 = 0.46$, $p < 0.0001$) and RL ($r^2 = 0.42$, $p < 0.003$). T_4 was at this time only affected by RL ($r^2 = 0.36$, $p < 0.0018$). T_3/T_4, on the other hand, was only affected by SS.

Use of the whole data set at either 0, 84, or 212 d in single regressions also showed that on day 0, T_3 levels were correlated with muscle protein content ($r^2 = 0.77$, $p < 0.04$). At day 84, plasma T_4 and T_3 were positively correlated with each other ($r^2 = 0.46$, $p < 0.003$) and with Bwt ($r^2 = 0.5$ and 0.46, $p < 0.0001$, and <0.0003, respectively). At day

212, plasma T_4 was correlated with HSI ($r^2 = 0.33$, $p < 0.0054$).

Discussion

Salmonids swim voluntarily for prolonged periods against water velocities below levels that lead to exhaustion, provided that their basal energy needs are satisfied (Brett et al. 1958). Sustained submaximal aerobic physical exercise has been reported to enhance fish growth (reviewed by Davison 1989). Contrary to what is known in mammals (reviewed by Goldspink 1985; Pette and Staron 1990), fish subjected to such exercise not only improve growth and feed efficiency, but also show marked myotomal hypertrophy in red, white, or both muscle types (e.g., Greer-Walker 1971; Greer-Walker and Pull 1973; Davison and Goldspink 1977; Greer-Walker and Emerson 1978; Johnston and Moon 1980; Totland et al. 1987) and enhanced protein synthesis in white somatic muscle (Houlihan and Laurent 1987). However, most of these studies also report an accelerated growth with increased SS. Naturally, accelerated growth will be associated both with elevated protein synthesis and muscle fibre hypertrophy, and the interdependence between these variables makes it unclear to what degree the reported effects on muscle protein synthesis and hypertrophy are related to SS or to a secondary consequence of accelerated growth.

Part of the benefits of sustained exercise on salmonid growth, observed in previous studies, may have resulted from holding control fish in quiescent water. However, fish stress may be greater in nonflowing water. In support of this, Boesgaard et al. (1993b) found that plasma cortisol levels were similar in rainbow trout forced to swim at 0.5 to 1.5 bl/s for 30 d, but the levels were elevated consistently in fish held in quiescent water. In the present study, we minimized differences in behavioral stress between control and exercised fish. To achieve this, we designed a circular swimming channel with no confinement of fish to any region, allowing fish to choose position in the current and to escape upstream or downstream in cases of aggression. The circular channel also permitted fish to reside in regions of lower water velocities (close to the sides of the channel) to repay any oxygen debt. This design minimized behavioral differences between fish swimming at different speeds but with some abatement in perfect laminar water flow.

Feed dispensed to a small region of a tank can increase the territorial behaviour of fish which in turn can lead to unequal food availability to all fish (Brännes and Alanärä 1993). This increases both feed waste and the size distribution of fish. In this study, the density of the feed pellets was selected to permit them to be carried once around the swimming channel before they touched bottom. Moreover, feed was dispensed continuously over several hours each day. Thus, the feeding protocol minimized territorial and feeding dominance behaviour between fish, and every fish had an opportunity to ingest the daily ration. The design of the experimental tank also enabled us to estimate the actual amount of food ingested, since the waste feed pellets could be counted with minimal disturbance to the fish. Other methods of ration estimation, e.g., hand feeding or the use of an inert marker (Talbot and Higgins 1983), involve repeated disturbance or handling of the fish.

In this study, SS between 0.5 and 1.5 bl/s did not affect growth, despite increased energy demands at high SS (Brett 1965; Brett and Glass 1973; Thorarensen et al. 1993). This elevated demand was offset by increased feed (energy) intake, reflected in a decreased feed efficiency. The elevated HSI observed at greater SS may be due to greater ration consumed at higher SS and thereby reflect an enhanced metabolic activity normally associated with increased nutrient utilization (Jobling 1985).

The extended range in SS used in the regression relating SS of individual tagged fish to their growth (0.3–1.9 bl/s) compared with the preset levels (0.5–1.5) used in the main effect model may explain why a significant negative relationship between SS and growth (SGR) is found in the former and not in the latter. This finding that a negative correlation exist between individual SS and SGR but not between preset SS and SGR indicates that a sustained swimming speed of 1.5 bl/s approached the maximum level tolerable for chinook salmon of this size.

Temporal changes in fish size and ration had the most influence on whole-body and muscle proximate composition of chinook salmon. We found no effect of SS on lipid content of either the fillet or the whole fish, indicating no shift in substrate utilisation from protein to fat. Such a shift was suggested for Arctic char (*Salvelinus alpinus*) by Christiansen et al. (1989). The relative increase in visceral tissue (CP) of fish at higher SS probably accounts for their elevated levels of whole-body protein because no effect of SS was found on muscle protein content. This elevated protein content of the viscera can be explained partially by the elevated HSI and an increased blood perfusion, and thereby higher blood content, in the digestive system of fish exposed to 1.5 bl/s compared with the fish exposed to 0.5 bl/s (Thorarensen et al. 1993).

Elevations in plasma thyroid hormone titres have been linked to enhanced somatic growth in salmonids (Higgs et al. 1982; Eales and MacLatchy 1989; Eales et al. 1992). Furthermore, T_4 is the main hormone secreted by the salmonid thyroid, and up to 70% of the plasma T_4 in trout may be deiodinated rapidly by 5′-monodeiodinase (5′D) extrathyroidally (e.g., in liver) to T_3, the more biologically active form of thyroid hormone (Higgs et al. 1982; Eales and MacLatchy 1989).

In this study, the consistent absence of a correlation between plasma levels of T_4 and T_3 coupled with weak temporal, SS, and RL effects on plasma T_4 levels, as compared with T_3, suggests that alterations in T_3 titres were caused by changes in 5′D activity and/or in plasma T_3 clearance and that T_4 availability was not necessarily determining the production of T_3. The consistent positive relationship between plasma T_3 levels and anabolic variables such as fish protein content (day 0), growth (day 84), and ration level (day 212) suggests that T_3 exerts an anabolic effect in seawater-adapted chinook salmon.

The ingestion of more food (protein and energy) in salmon swimming at higher speeds on maximum ration can partly explain the elevation in plasma T_3 (Eales 1988; Eales et al. 1992). However, SS also influenced the response. When the swimming variables (preset SS, individual SS, and water velocity) and feed-related variables (RL, individual SGR, and individual total feed ingested (calculated as individual growth divided by the mean feed efficiency)) were arranged in order of the degree to which they accounted for variation in plasma T_3 levels (SAS stepwise procedure, $p < 0.05$), it was found that water velocity and then RL were the variables best explaining the model.

In conclusion, sustained exercise did not enhance chinook salmon growth despite increased appetite. The elevated energy intake in fish swimming at higher speeds was presumably necessary to satisfy the increased energy requirements. Within the limits of this study, we found that a combination of full ration and low SS (0.5 bl/s) provides the most cost-effective protocol for farming chinook salmon. However, the optimal SS will most likely vary both with the size of the fish, fish species, temperature range, and the type of culture system used (Brett and Glass 1973).

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Changes in Serum Free Thyroxine, Prolactin, and Olfactory Activity during Induced Smoltification in Atlantic Salmon (*Salmo salar*)

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Serum levels of free thyroxine (FT_4) and prolactin (PRL) and olfactory activity were studied in juvenile Atlantic salmon (*Salmo salar*) induced to undergo premature smoltification by a regime of increasing temperature and photoperiod. The olfactory epithelium was stimulated with L-alanine or taurocholate (10^{-8}–10^{-4} M) while electroencephalographic responses were recorded from the rostrolateral and rostromedial surfaces of the olfactory bulb. Two peaks of olfactory activity occurred during the 8-wk test period for three combinations of recording site and odorant, lateral–alanine, lateral–taurocholate, and medial–taurocholate, but not for the medial–alanine combination. The first peak occurred at the beginning of smoltification (the "acute phase") coinciding with the previously described sensitive period for olfactory imprinting. Whereas this peak of olfactory activity was not associated with a rise in serum FT_4, the second peak, occurring after the acute phase of smoltification, coincided with a FT_4 peak. PRL remained constant throughout the acute phase of smoltification but increased 1 wk after the second peak of olfactory activity. It is concluded that olfactory activity is at least partly related to thyroid activity during induced smoltification.

Les teneurs sériques de thyroxine libre (FT_4) et de prolactine (PRL) sont examinées en même temps que l'activité olfactive chez de jeunes saumons atlantiques (*Salmo salar*) au cours d'une « smoltification » prématurée et induite par un régime croissant de température et de photopériodes. L'épithélium olfactif est stimulé à la L-alanine ou à la taurocholate (10^{-8} à 10^{-4} M), alors que les réponses électroencéphalographiques sont enregistrées à la surfaces des régions rostrolatérales et rostromédiales du bulbe olfactif. Au cours de 8 sem d'essais, deux pics d'activité olfactive sont mis en évidence pour trois combinaisons de sites d'enregistrement et de substances odorantes, soit les combinaisons latéral–alanine, latéral–taurocholate et médial–taurocholate, mais non pour la combinaison médial–alanine. Le premier pic est apparu au début du processus de smoltification (« phase aiguë »), concomitamment à la période sensible d'empreinte olfactive, décrite dans le cadre d'études antérieures. Bien que ce pic d'activité olfactive ne soit pas relié à une hausse de FT_4, le deuxième pic apparaissant après la phase aiguë de la smoltification coïncidait à un pic de FT_4. Le taux de PRL est demeuré constant pendant toute la phase aiguë de la smoltification mais a augmenté 1 sem après le deuxième pic d'activité olfactive. Nous concluons que l'activité olfactive est au moins partiellement reliée à l'activité thyroïdienne lors de la smoltification induite.

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Thyroid hormones participate in various metabolic and developmental events in the smoltification of anadromous salmonids (see review by Dickhoff and Sullivan 1987) and may have the primary role of preadapting fish to exploit new food sources in the marine environment (Specker 1988). Thyroid hormones may promote high hyposmoregulatory ability in smolts at the time of seaward migration (Grau et al. 1985; Rydevik 1990) to maximize seawater survival. The response of the juvenile salmon thyroid to seawater is related to development (Specker and Kobuke 1987). River-dwelling parr prematurely transferred into seawater before the smolt stage become hypothyroidal dwarfs or stunts (Clarke and Nagahama 1977; Bern 1978; Young et al. 1989b).

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One developmental role of thyroid hormones at the time of smoltification may be to prepare juvenile salmon for their future reproductive migration towards their ancestral spawning grounds. According to the imprinting hypothesis (Hasler and Scholz 1983), juvenile anadromous salmonids learn the odoriferous features of their natal river and use this information for recognizing their homestream odor as homing adults. Scholz (1980) hypothesized that thyroid hormones may activate the brain during smoltification to facilitate olfactory imprinting. Evidence of a thyroid-imprinting interaction (Hasler and Scholz 1983) includes (1) successful imprinting by juvenile coho salmon (*Oncorhynchus kisutch*) to synthetic chemicals when blood levels of total thyroxine (T_4) reach peak values as compared with fish before the hormonal surge and (2) successful imprinting in juvenile coho salmon injected with thyrotropin (TSH) while exposed to synthetic chemicals before smoltification as compared with fish injected with adrenocorticotropin (ACTH) or with the solvent.

Morin et al. (1989a) reported two peaks of odor learning during smoltification, but only the first one was associated with odor memory. The interval of the first learning-memory peak was considered as the sensitive period for olfactory imprinting (SPOI) in Atlantic salmon (*Salmo salar*). In another study, Morin et al. (1989b) showed that histological indices of thyroid activity, epithelial cell height and follicular eccentricity, increase to peak values during the SPOI, while plasma level of T_4 does not increase concomitantly, and they speculated that rapid blood-tissue (e.g., brain) fluxes of thyroid hormones could facilitate imprinting.

The above studies suggest a thyroid–brain interaction during a smoltification-associated SPOI, but none has provided neural evidence for such interaction. Thus, for the first time in an anadromous salmonid, we recorded electroencephalographic (EEG) responses to odorants from the rostromedial and rostrolateral surfaces of the olfactory bulb and determined the relationship between serum levels of free T_4 (FT_4) and olfactory activity at the time of induced smoltification. This is also a first attempt to study the relationship between FT_4 and olfactory functions in fish. We also measured serum prolactin (PRL) to provide some comparisons with a smoltification-associated hormone thought to be unrelated to olfactory functions.

Materials and Methods

Fish

We used 2-yr-old juvenile Atlantic salmon which at the beginning of the study had a mean fork length of 11.3 cm ± 0.7 SD. They were hatchery-raised offspring of a wild stock obtained from "Lierelva", a small river 30 km southwest of Oslo, Norway. Fish were kept, 50 in each of six identical 1-m-diameter (450-L) tanks supplied with dechlorinated water (6.4 ± 0.5°C), under a constant 8 h light : 16 h dark photoperiod regime (light = 08:00–16:00). Light intensity was kept constant but varied from 1 to 30 lx depending on the position in the tanks. The mean water flow was 4.9 L/min. Fish were not fed during the initial 16-d acclimation period. After acclimation, fish were fed until satiation with dry food pellets (ST40-31 Ewos). Feeding occurred twice daily (9:00 and 14:00) during the first week following acclimation and three times daily (9:00, 14:00, and 17:00) during the 8-wk test period.

Smoltification

To induce smoltification the water temperature was raised to 12.6°C (range = 11.6–13.6°C) and the day length increased to 16 h light : 8 h dark at the beginning of week 1 (light = 08:00–24:00). These conditions were maintained during the 8-wk test period. On the first day of week 1, water temperature was increased slowly to 12.6°C between 9:00 and 12:00. On the same day, the 8-h light increment began at 16:00 so that daylight persisted until 24:00.

Using external morphological characteristics, fish were eventually classified as parr, smolt, or desmolt (Morin et al. 1989a). Parr were characterized by the presence of vertical parr marks, the contrasting dark back and yellowish belly, red spots near the lateral line, and opercular black spots on either side of the fish. Smolts were characterized by the presence of blackened pectoral fins and caudal fin. Fish were designated desmolts following the reappearance of the contrasting dark back and yellowish belly and vertical parr marks. Desmolts are fish that have undergone a process of desmoltification because they have been prevented from migrating to sea after smoltification (Evropeytseva 1963).

Experimental Design

Fish were sampled from all tanks each week, and one fish per day was taken for EEG recordings. Fish were tested at 09:30 and blood samples were taken after the EEG recordings at 12:00. EEG recordings were made during eight successive weeks of 5 d. In total, 40 fish were tested for EEG, but data on only 36 of them are reported in this study because sufficient serum for FT_4 and PRL determination could not be obtained for the other four.

Testing Procedure

Fish were tranquilized for 2.5 min in chlorobutanol (80 mg/L), anesthetized intraperitoneally with amytal (30 mg/kg) (Keys and Wells 1931), paralyzed with tubocurarin chloride (2.5 mg/kg), measured, weighed, and noted for their external morphological characteristics. Fish were wrapped in a wet cloth and maintained in a fish-holding cradle with the head fixed under continuous perfusion of the gills (0.3 L/min) with dechlorinated tap water (mean temperature = 9.1°C ± 1.5 SD). The skull roof was opened and mesenchymal tissue removed to expose the dorsal surface of the two olfactory bulbs. The right olfactory epithelium was continuously perfused with standardized artificial freshwater (Thommesen 1982). This rinsing solution contained 0.4 mM Na^+, 0.04 mM K^+, 0.4 mM Ca^{2+}, 0.84 mM Cl^-, and 0.4 mM HCO_3^- (pH = 7.6). Nasal water flow was kept constant at 6.6 ± 0.2 mL/min.

Anhydrous L-alanine (Sigma Chemical Co.) and taurocholic acid sodium salt (taurocholate; Sigma Chemical Co.) were diluted in artificial freshwater and used as odorants. Each odorant was prepared in decadic aliquots, from 10^{-8} to 10^{-4} M. During odor stimulation, the odorant drained from a 10-mL glass pipette through the continuous flow of rinsing water, and this flowing stimulus was manually controlled by a three-way stopcock.

The order of presentation of L-alanine or taurocholate varied at random. Each fish was presented with each odorant in ascending concentrations. Three consecutive trials occurred at each concentration of a given odorant. By using rinsing water as a control stimulus, three other consecutive

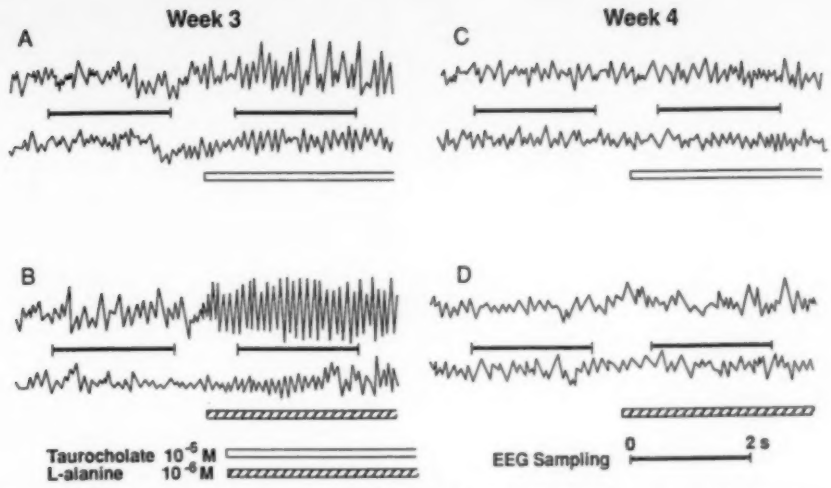

FIG. 1. Examples of computer-stored EEG signals redrawn with the Macintosh's Aldus Freehand software. For each combination of recording site and odorant, 2-s EEG samples were taken as indicated before and during stimulation of the olfactory epithelium with an odorant (10^{-6} M) to calculate energy ratio (energy value after/energy value before) in Atlantic salmon at the time of smoltification. (A and C) EEG signals during weeks 3 and 4 for the lateral–taurocholate (upper signals) and medial–taurocholate combinations (lower signals); (B and D) EEG signals during weeks 3 and 4 for the lateral–alanine (upper signals) and medial–alanine combinations (lower signals).

trials took place between the first and second odorant. Before the onset of a trial at a given concentration of odorant, the glass pipette was rinsed twice with that concentration before it was filled with 10 mL of the odor solution. A trial began by switching on the three-way stopcock for 4 s until 2 mL of the odor stimulus had drained from the pipette to reach the fish's right naris. Then the three-way stopcock was switched off until the next trial. A 60-s interval was used to avoid adaptation between trials.

EEG recordings and stimulation of the olfactory organ were made on the right side. Responses were recorded from two different positions simultaneously: one recording electrode was placed at the rostrolateral region of the bulb and the other at the rostromedial region. A preliminary experiment established that each of these two distinct topographical positions corresponded to an optimal and specific EEG response to L-alanine and to taurocholate, respectively. In other salmonids, EEG responses to amino acids were also centered on the rostrolateral region of the olfactory bulb whereas responses to bile salts were centered mainly on the rostromedial region (Thommesen 1978; Døving et al. 1980).

In the present study, we used four different combinations of recording site and odorant: rostrolateral–alanine, rostromedial–alanine, rostrolateral–taurocholate, and rostromedial–taurocholate. To simplify, we called these combinations lateral–alanine, medial–alanine, lateral–taurocholate, and medial–taurocholate. Potentials were registered by two conventional Ag/AgCl electrodes coated with an insulating layer of varnish, leaving a 1-mm uncoated tip. The tip diameter of each electrode was 105 μm. It was positioned on the dorsal surface of the bulb.

Potential changes were fed into two differential preamplifiers with frequency response 0.1–30 Hz, displayed on an oscilloscope, and stored on a tape recorder (Tandberg FM, series 100; Dc-1250 Hz) and a pen recorder. Recordings from the rostromedial or from the rostrolateral region of the bulb were monopolar, and the preparation was grounded through the common indifferent electrode which was placed on the chondrocranium just adjacent to the opening in the skull. Because the reference electrode was grounded, the recordings were actually single ended.

Blood flow in the brain surface vessels was inspected regularly to ensure adequate circulation. Tissue paper was used as a wick to remove the exuding fluid covering the bulb in the vicinity of the recording electrodes.

Blood Sampling

Following the EEG recordings, fish were removed from their holding cradle and blood drawn from the caudal blood vessels. The blood was allowed to clot at 4°C overnight before centrifugation, and serum aliquots were stored at −18°C until assayed for FT_4 and PRL. All samples were measured in duplicate and were run in the same assay series.

Determination of FT_4 by Immunofluorescence

The concentrations of FT_4 in the serum were measured with a nonisotopic dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA FT_4 kit, Wallac Chemical Laboratories, Finland). The development of europium labelling reagents and a fluorescence-detection method based on europium dissociation and the principle of time-resolved fluorometry have been described in detail previously (Hemmilä et al. 1984).

The detection limit defined as the value which is 2 SD below the "zero standard" values was approximately 2 pmol/L. The intraassay coefficient of variation was less than 5% for values below 40 pmol/L.

PRL Determination by Radioimmunoassay

The iodination of salmonid PRL by the lactoperoxidase technique and the PRL radioimmunoassay procedure were described by Andersen (1989). The detection limit was

TABLE 1. Number of Atlantic salmon used in this study classified as parr, smolt, or desmolt based on external morphological characters (Morin et al. 1989a). Fish were the hatchery-raised offspring of a wild stock from "Lierelva", Norway. Number of fish discarded because of insufficient blood supply shown in parentheses.

	Weeks of induced smoltification							
Stage	1	2	3	4	5	6	7	8
Parr	2 (3)	5	3 (1)	1	1	0	1	0
Smolt	0	0	1	4	4	3	2	3
Desmolt	0	0	0	0	0	2	2	2

approximately 6 pmol/L, and the intraassay coefficient of variation was 3%.

Data Treatment

Response magnitudes to odor stimulation were measured from the electric potentials recorded on magnetic tape. The signals were digitized by an analog/digital converter and stored in a microcomputer. The "energy" was operationally defined as the sum of the amplitudes squared with a sampling rate of 1 kHz. To assess the EEG response following stimulation, the "energy ratio" was automatically calculated in the computer by dividing the relative energy value for a 2-s window 0.5 s after the onset of odor presentation by the energy value for a 2-s window 0.5 s before the odor presentation (see Fig. 1). An energy ratio inferior or equal to 1.0 indicated no "response".

Statistical Analyses

Values of FT_4 were log transformed to fit the normal distribution curve. Instead of using a one-way analysis of variance to compare overall differences in energy ratio between weeks, for each combination of recording site and odorant as well as for levels of FT_4 and PRL, we have made a priori nonorthogonal comparisons between successive weeks (Kirk 1982). An analysis of variance may become too conservative when the number of weeks being compared increases. Week 1 was not considered in these analyses, since there were only two subjects. One-way analyses of variance were only used to compare energy ratios, levels of FT_4 or PRL between parr, smolts, and desmolts, or relative energy values (preceding odor presentations) between weeks. A multiple regression was used to evaluate the amplitude of the correlation between levels of FT_4 or PRL and the energy ratio in the four combinations of recording site and odorant. The Pearson product-moment correlation was used to examine the relationship between levels of FT_4 and PRL.

Results

Smoltification

We propose the use of the expression "latent phase" of smoltification to describe the initial period when physiological changes may occur in fish before the appearance of external signs which would classify them as smolts. The latent phase of smoltification occurred during weeks 1 and 2 because no fish had become smolts (Table 1). We also propose the use of the expression "acute phase" of smoltification to describe the period beginning with the appearance of a number of external signs to classify fish smolts and ending with the reappearance of parr coloration to classify fish desmolts. The acute phase of smoltification began during week 3 when one quarter of the fish became smolts and ended during week 5 when four fifths of the fish were smolts. Fish began to show signs of desmoltification during week 6 as two fifths of the fish had become desmolts. One fish remained a parr during week 7.

Olfactory Activity

For any recording site, the EEG responses to 10^{-6} M L-alanine or taurocholate were generally similar to the responses obtained at the other concentrations of odorants (Fig. 2). However, there were some inconsistencies in the data. For example, responses in the lateral–alanine combination were concentration related during weeks 3 and 4 but more variable during other weeks. Two peaks of olfactory activity occurred in the lateral–alanine, lateral–taurocholate, and medial–taurocholate combinations, but no peaks occurred in the medial–alanine combination (Fig. 3). The first peak of olfactory activity occurred during week 3 (within the acute phase of smoltification) whereas the second peak occurred during week 7 (after the acute phase of smoltification). Most significant differences in energy ratio between consecutive weeks were associated with the first peak of olfactory activity. There was a significant difference in energy ratio between weeks 3 and 4 for the lateral–alanine, lateral–taurocholate, and medial–taurocholate combinations ($P < 0.05$). The greater energy ratio occurring during week 3 than during week 4 was the result of original (energy-converted) EEG signals of greater amplitudes following stimulation of the olfactory epithelium with L-alanine or taurocholate (Fig. 1). Prior to stimulation, EEG signals were relatively stable. There were also significant differences in energy ratio between weeks 6 and 7 in the lateral–alanine combination ($P < 0.05$) and between weeks 4 and 5 in the medial–alanine combination ($P < 0.05$) (Fig. 3). There were no differences in the relative energy values preceding odor presentations.

Serum FT_4 and Serum PRL

Significant differences in the levels of FT_4 between weeks 6 and 7 ($P < 0.05$) (Fig. 3A) and in the levels of PRL between weeks 7 and 8 ($P < 0.01$) (Fig. 3D) were found. Using the Pearson product-moment correlations, there was no correlation between FT_4 and PRL ($P = 0.39$).

Serum FT_4, Serum PRL, and Olfactory Activity

There were no differences in energy ratio or in the levels of FT_4 or PRL between parr, smolts, and desmolts. Using multiple regression analysis, a significant proportion of the variance of FT_4 could be explained by the lateral–taurocholate ($P < 0.05$), lateral–alanine ($P < 0.05$), or medial–taurocholate combination ($P < 0.05$), but this was not the case with the medial–alanine combination. Together, the four combinations of recording site and odorant could not explain a significant proportion of the variance of FT_4. However, with a second multiple regression analysis of FT_4 on the lateral–taurocholate, lateral–alanine, and medial–taurocholate combinations, the regression equation was significant ($F = 3.78$, df = 2,33; $P < 0.05$). A multiple regression analysis of PRL on the four combinations of recording site and odorant revealed no relationship between PRL and olfactory activity.

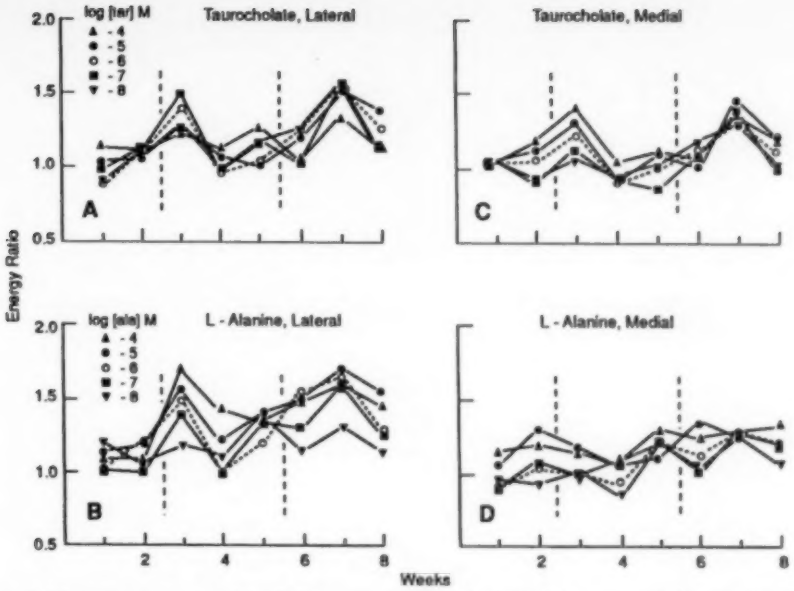

FIG. 2. Mean energy ratio of the EEG responses to L-alanine (ala) or taurocholate (tar) at different concentrations (10^{-8} to 10^{-4} M) over the 8 wk of smoltification in Atlantic salmon. (A) Lateral–taurocholate combination; (B) lateral–alanine combination; (C) medial–taurocholate combination; (D) medial–alanine combination (SEMs omitted for clarity). The area between the vertical broken lines delimits the acute phase of smoltification.

Discussion

Our results demonstrate that olfactory activity is at least partly related to thyroid activity. We found two peaks of olfactory activity at the time of smoltification, but only the second peak corresponded to an increase in serum FT_4 (week 7). The first peak during week 3 was not associated with any increase in FT_4. These results are similar to those of Morin et al. (1989b) using a comparable procedure to induce smoltification in Atlantic salmon. They reported a first peak of olfactory learning with no increase in plasma T_4 during week 3 whereas a second peak was associated with a significant increase in T_4 during week 6.

The two peaks of olfactory activity did not appear in all four combinations of recording site and odorant. This suggests that a spatial distribution of the EEG response exists across these combinations. The lateral EEG response to L-alanine was apparently greater than the medial EEG response to L-alanine, especially during weeks 3 and 7. Such spatial distribution of the EEG response is also found in Arctic char (*Salvelinus alpinus*), Arctic grayling (*Thymallus thymallus*), and brown trout (*Salmo trutta*) when the olfactory epithelium is stimulated with an amino acid (Thommesen 1978; Døving et al. 1980).

The lateral EEG response to taurocholate was apparently similar to the medial EEG response to taurocholate. This is different from what has been found in other salmonids. Døving et al. (1980) reported strong medial EEG responses to taurocholate, but hardly any lateral EEG response to taurocholate. The difference in spatial distribution of the EEG response may reflect plasticity of the neuronal organization in the olfactory bulb. Other aspects of olfactory activity in Atlantic salmon during smoltification are discussed elsewhere (Morin and Døving 1992).

Although the two peaks of olfactory activity or learning have been reported repeatedly in Atlantic salmon during photoperiodically induced smoltification (Morin et al. 1989a, 1989b; Morin and Døving 1992), the possibility that nonbiological factors such as changes in water quality could have generated these increments cannot be ruled out totally. One way to verify this would be to compare groups of fish whose induction of smoltification begins at different times in order to test the hypothesis that the peaks of olfactory activity always occur at the same time intervals from the onset of smoltification.

Each of the two distinct topographical positions used in this study, rostrolateral and rostromedial, corresponded to an optimal and specific EEG response to L-alanine and to taurocholate, respectively. For practical reasons, we did not search for other regions on the olfactory bulb. In rainbow trout (*Oncorhynchus mykiss*), Hara et al. (1973) compared EEG recordings from different regions of the olfactory bulb and found greater responses from the posterior than the anterior (rostral) region following stimulation of the olfactory epithelium with an amino acid (L-serine).

Our values of serum levels of FT_4 are 10 times lower than the values reported in Atlantic salmon by Boeuf et al. (1989). However, these authors did not measure the absolute levels of FT_4 but instead used an index, precluding direct numerical comparison. Potentially stress-induced procedures such as those used in our EEG studies do not affect the levels of serum FT_4 and PRL. Similar levels of these hormones are found in fish from which blood is removed shortly after tranquilization with chlorobutanol (P.-P. Morin, unpublished data). Contrasting with most previous salmon studies, we measured FT_4 instead of T_4. In serum, FT_4 exists in equilibrium with protein-bound T_4, and in Atlantic salmon it has been shown that seasonal changes in FT_4 are highly

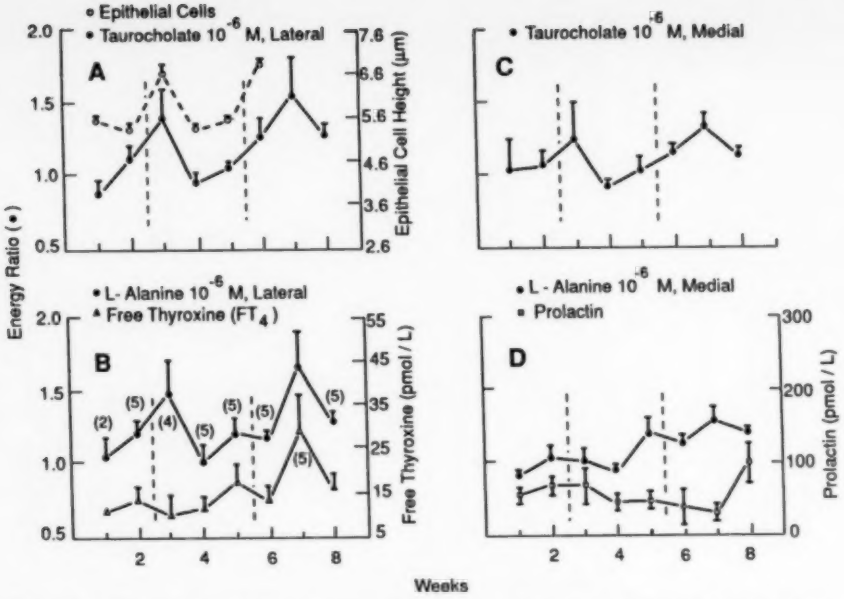

FIG. 3. Variations in physiological parameters in Atlantic salmon at the time of smoltification. The area between the vertical broken lines delimits the acute phase of smoltification. Each point is the mean (±SEM) and *n* values are shown in parentheses. (A) Energy ratio (olfactory activity) for the lateral–taurocholate combination (for the purpose of the discussion, a curve drawn from Morin et al. (1989b) also shows changes in the thyroid epithelial cells during induced smoltification); (B) serum free thyroxine (FT_4) and energy ratio for the lateral–alanine combination; (C) energy ratio for the medial–taurocholate combination; (D) serum prolactin and energy ratio for the medial–alanine combination.

correlated with changes in T_4 (Boeuf et al. 1989). The profile in FT_4 corresponds exactly to the profile in T_4 reported by Morin et al. (1989b). We believe that FT_4 is a better indicator of thyroid activity on target cells than T_4. The T_4 molecules bound to proteins are unavailable for biological action whereas the FT_4 molecules are free to leave the capillaries and reach the target cells. Moreover, the amount of T_4 bound to proteins depends on the concentration of serum proteins and the binding affinities of those proteins. It follows that protein-bound (total) T_4 does not necessarily reflect the concentration of the biologically active free fraction.

The first peak of olfactory activity occurring during week 3 of induced smoltification is associated with a period of apparent high thyroid activity, although there are no changes in serum FT_4 at that time. Our data show that the first peak of olfactory activity (Fig. 3) coincides with increased epithelial cell height reported earlier by Morin et al. (1989b), suggesting that heightened olfactory activity occurs during a period of high production in T_4. At that time, these follicular hormones appear to be rapidly evacuated towards the bloodstream. The colloid of hyperactive follicles loses its intensity in staining reaction or is completely withdrawn (P.-P. Morin, unpublished data). Hoar (1939) also observed a withdrawal of the colloid in extremely active follicles when Atlantic salmon underwent smoltification.

One hypothesis explaining unaltered serum FT_4 during week 3 of induced smoltification when thyroid histological activity appears high may be a greater blood–tissue FT_4 flux (net movement) at the beginning of smoltification than at the end. Specker et al. (1984) provided substantial evidence for such a mechanism in juvenile coho salmon by studying T_4 metabolism at the beginning of smoltification, before plasma T_4 normally reached peak values, and after smoltification, at the termination of the T_4 elevation. They found that fish at the beginning of smoltification as compared with fish after smoltification had a threefold greater T_4 production rate, a fourfold greater distribution of T_4 in the tissues, and a twofold greater clearance rate of T_4 from the tissues.

In addition to preventing a rise in plasma T_4, a great blood-tissue T_4 flux at the beginning of smoltification may allow large quantities of T_4 to accumulate in tissues such as the brain. Consistent with this view are the recent findings of Specker et al. (1992) in coho salmon. In their study, the brain content in T_4 increased significantly and reached its highest level at the beginning of smoltification, while plasma T_4 remained constant. This asynchrony of changes in the brain and plasma T_4 occurred 3 wk before the peak in plasma T_4. Higher levels of T_4 in the brain of salmon at the beginning of smoltification may optimize the conversion of local T_4 into 3,5,3′-triiodo-L-thyronine (T_3) by the 5′-monodeiodinase (5′D) enzyme. The outer-ring monodeiodination of T_4 by the 5′D enzyme in the brain appears to be an important source of brain T_3 in Atlantic salmon during smoltification (Morin et al. 1993). The low K_m of this enzyme in the brain of Atlantic salmon (Morin et al. 1993) is similar to that of the type II T_4-5′D enzyme reported in the brain of mammals (Leonard and Visser 1986), where it is believed to produce T_3 primarily to occupy T_3 brain receptors (Crantz et al. 1982). Specker et al. (1992) reported an eightfold greater T_3/T_4 ratio in the brain of coho salmon at the beginning of

smoltification, while plasma T_4 remained constant. More T_3 may be required at the beginning of smoltification to maximize the binding of T_3 to putative nuclear receptors in the brain.

The olfactory activity peak of week 3 may be associated with great plasma–tissue fluxes of thyroid hormones and may occur during SPOI, the sensitive period for olfactory imprinting as described by Morin et al. (1989b). As suggested earlier for coho salmon (Scholz 1980; Hasler and Scholz 1983), more T_3 may interact with the receptors of target cells in the brain to induce neuronal proliferation during olfactory imprinting. Such thyroid–brain interaction could facilitate memory encoding of biologically significant odor signals in the context of imprinting. Saturable, high-affinity T_3 receptors are present in the brain of salmonids (Scholz et al. 1985; Bres and Eales 1988; White et al. 1990), and elevated T_3 levels are found in their brain at the beginning of smoltification (Brown et al. 1989). Specific enzyme activities regulating T_3 formation and T_3 degradation in the brain of Atlantic salmon during smoltification may provide fine control of intracellular T_3 availability to receptor sites (Eales et al. 1993). Some affinity of nuclear T_3 receptors for T_4 has been reported in salmonids (Darling et al. 1982; Bres and Eales 1986) even though T_3 is thought to be the active hormone at the cellular level (Eales 1985).

The second peak of olfactory activity occurred after the acute phase of the smoltification process and coincided with a peak in serum FT_4 to suggest a relationship with thyroid activity. This rise in serum FT_4 is similar to that of plasma T_4 typically observed in anadromous salmonids during smoltification (Hoar 1988). It is indicative of heightened thyroid activity rather than a reduced activity in the 5′D enzyme that converts T_4 to T_3. During smoltification the activity of this enzyme is positively correlated with plasma T_4 (Morin et al. 1993). Under conditions of extended photoperiod, the T_4 (or FT_4) peak occurs at the end of smoltification (Morin et al. 1989b) or shortly after, as in the present study. The thyroxine surge delay is attributable to the acceleration of parr–smolt development under conditions of extended photoperiod.

The second peak in serum FT_4 apparently occurred during an interval of high ability for odor learning but after the smoltification-associated SPOI as described in Atlantic salmon by Morin et al. (1989a, 1989b). Smolts retain some learning ability for new odors shortly after smoltification (Morin et al. 1990). The rise in serum FT_4 during the second peak of olfactory activity may indicate a lesser blood–tissue flux of FT_4 than that associated with the first peak of olfactory activity. A reduced flux of T_4 during the second peak of olfactory activity may only affect the sensitivity of the olfactory system to odorants to increase learning ability.

PRL did not change significantly in the course of smoltification. No relationship was found between serum levels of PRL and FT_4 or between PRL and olfactory activity, suggesting that PRL had no effect on olfaction and olfactory imprinting during smoltification. However, PRL increased significantly during week 8 when several fish had become desmolts. Significant elevation of PRL levels has also been reported in Atlantic salmon kept in freshwater during smoltification (Prunet and Boeuf 1989; Prunet et al. 1989). In some studies, PRL has also been shown to increase significantly as fish become desmolts (e.g., Young et al. 1989a). PRL exerts a variety of functions in vertebrates, but its role in teleosts appears predominantly to maintain ionic and water balance in freshwater (see Clarke and Bern 1980). In anadromous salmonids, PRL increases dramatically when postsmolts are transferred from salt- to freshwater (Fargher and McKeown 1990), thus confirming the freshwater-adapting role of this hormone. A PRL elevation would be beneficial for desmolts, since they must readapt to freshwater for survival.

We conclude that (1) at least a partial relationship exists in Atlantic salmon between olfactory and thyroid activities during induced smoltification, (2) a hypothesized greater blood–brain flux of FT_4 during the peak of olfactory activity may facilitate memory encoding in the context of odor imprinting (SPOI), and (3) a hypothesized lesser blood–brain flux of thyroid hormones during the second peak of olfactory activity may only increase learning ability by increasing olfactory sensitivity to odors.

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Influence of Larval Gizzard Shad (*Dorosoma cepedianum*) Density on Piscivory and Growth of Young-of-Year Saugeye (*Stizostedion vitreum* × *S. canadense*)

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Stahl, T.P., and R.A. Stein. 1994. Influence of larval gizzard shad (*Dorosoma cepedianum*) density on piscivory and growth of young-of-year saugeye (*Stizostedion vitreum* × *S. canadense*). Can. J. Fish. Aquat. Sci. 51: 1993–2002.

Growth and survival of young-of-year saugeye (*Stizostedion vitreum* ♀ × *S. canadense* ♂) (stocked into Ohio reservoirs to create sport fisheries) are probably influenced by prey availability, variations in which may account for historically documented variability in stocking success. Because saugeye switch from a diet of zooplankton to fish once stocked, we sought to determine experimentally if saugeye size and available ichthyoplankton, i.e., larval gizzard shad (*Dorosoma cepedianum*), affected this switch and whether piscivory improved saugeye growth. In an enclosure experiment, saugeye (33.9 mm TL) immediately switched to piscivory when exposed to ichthyoplankton densities of 20 and 100·m^{-3}, growing faster when more gizzard shad were available. In another enclosure experiment, saugeye 30–49 mm TL consumed 14-mm gizzard shad. In ponds (N = 4 ponds·treatment^{-1}) containing zooplankton and chironomids, we compared saugeye growth with and without larval gizzard shad and found, as in the first enclosure experiment, that piscivory improved saugeye growth. Neither saugeye size nor ichthyoplankton density influenced how quickly saugeye switched to piscivory. We conclude that managers should stock saugeye ≥30 mm 1–2 wk before peak ichthyoplankton densities to improve saugeye growth and survival by enhancing opportunities for exploitation of young-of-year gizzard shad.

La croissance et la survie de jeunes de l'année issus du croisement entre le doré jaune et le doré noir (*Stizostedion vitreum* ♀ × *S. canadense* ♂) (ensemencés dans des réservoirs en Ohio pour la pêche sportive) sont probablement influencées par l'abondance des proies, dont les variations peuvent expliquer la variabilité historique du succès de l'ensemencement. Étant donné que ce doré hybride passe d'un régime formé de zooplancton à un régime piscivore dès l'ensemencement, nous avons tenté de déterminer expérimentalement si la taille du poisson et la présence d'ichtyoplancton, c.-à-d. la larve de l'alose à gésier (*Dorosoma cepedianum*), influençaient cette modification du régime et si le régime piscivore accélérait la croissance du doré. Dans une expérience en enclos, les dorés (LT = 33,9 mm) ont adopté immédiatement un régime piscivore lorsqu'ils ont été exposés à des densités d'ichtyoplancton de 20 et de 100·m^{-3}, et leur croissance s'est révélée plus rapide en présence d'une plus grande quantité d'aloses à gésier. Dans une autre expérience en enclos, des dorés de 30 à 49 mm de LT ont consommé des aloses à gésier de 14 mm. Dans des étangs (N = 4 étangs·traitement^{-1}) renfermant du zooplancton et des chironomides, nous avons comparé la croissance du doré avec et sans la présence d'aloses à gésier. Nous avons observé, comme dans la première expérience en enclos, que le régime piscivore activait la croissance des dorés. Ni la taille des dorés, ni la densité de l'ichtyoplancton n'influençaient la rapidité avec laquelle les dorés adoptaient le régime piscivore. Nous concluons qu'il faut introduire ce doré hybride lorsqu'il mesure ≥ 30 mm et 1 à 2 sem avant que la densité d'ichtyoplancton n'atteigne son maximum de manière à accélérer la croissance et la survie du doré en améliorant sa capacité d'exploiter les aloses à gésier de l'année.

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Saugeye (*Stizostedion vitreum* ♀ × *S. canadense* ♂) are predators stocked annually as young-of-year (YOY) into Ohio reservoirs to create and maintain sport fisheries. Survival of this hybrid varies dramatically among reservoirs and across years at the same reservoir (Stahl et al. 1992, 1993; M. Austin, Ohio Division of Wildlife, 1076 Old Springfield Pike, Box 576, Xenia, OH 45385, personal communication). In YOY fishes, growth strongly influences survival through the first growing season (Gutreuter and Anderson 1985; Miller et al. 1988; Madenjian and Carpenter 1991; Madenjian et al. 1991) and first winter (Oliver et al. 1979; Adams and DeAngelis 1987; Post and Evans 1989). Therefore, conversion to piscivory by YOY fishes may be particularly critical to success because feeding on fish increases condition, growth, and survival as compared with the use of other prey (Hokansen and Lien 1986; Wicker and Johnson 1987; Buijse and Houthuijzen 1992).

Percids reared in hatchery ponds are initially planktivorous, become benthivorous (Fox 1989; Fox et al. 1989; Culver et al. 1992; Madon and Culver 1993), and finally switch to piscivory if fish prey are available (Mathias and Li 1982). In Ohio reservoirs, larval gizzard shad (*Dorosoma cepedianum*)

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are the primary prey (B.M. Johnson et al. 1988; Bremigan et al. 1991; Stahl et al. 1992) available to stocked saugeye. In fact, gizzard shad are preferred prey for many predators (Noble 1981; Carline et al. 1986; Matthews et al. 1988; Wahl and Stein 1988), including YOY saugeye (B.L. Johnson et al. 1988; B.M. Johnson et al. 1988; Stahl et al. 1992). Conceivably, high densities and preferred status of gizzard shad should permit rapid saugeye growth if they are stocked into systems with larval gizzard shad. However, saugeye often delay switching from planktivory and benthivory to piscivory once stocked (Stahl et al. 1992; Stahl 1993). This delay is unexpected given the benefit piscivory should convey.

One factor that could affect the switch from one prey type to another is prey density (Mittelbach 1981; Werner and Mittelbach 1981; Pyke 1984; Carline et al. 1986; Lyons 1987) — variations which could modify encounter rates between predators and their prey. As Mathias and Li (1982) have suggested for walleye and larval fish (i.e., ichthyoplankton), density likely also affects the time to switch for saugeye.

Another factor that may affect the switch to piscivory by saugeye is saugeye size in relation to gizzard shad size. Generally stocked into Ohio reservoirs at 30–50 mm (Stahl et al. 1992), saugeye (Lynch et al. 1982; B.L. Johnson et al. 1988) and walleye (Smith and Pycha 1960; Parsons 1971; Mathias and Li 1982; Jackson et al. 1992) are piscivorous at ≥39 and ≥34 mm, respectively, on fish 33–50% of their total length (Parsons 1971; Knight et al. 1984; B.M. Johnson et al. 1988). Therefore, some saugeye stocked into Ohio reservoirs may either be too small to switch to fish prey or they may not find sufficient prey fish of appropriate size.

Once an age-0 fish becomes piscivorous, its growth and survival are dictated by the availability of age-0 forage fishes (Smith and Pycha 1960; Forney 1976; Carlander and Payne 1977; Adams et al. 1982; Carline et al. 1986; Persson and Greenberg 1990). In Ohio reservoirs, larval gizzard shad densities at the time of saugeye stocking are positively related to saugeye weight in the fall and percent survival through the fall (Stahl 1993). Initial ichthyoplankton availability when stocked may be important for saugeye growth and survival, just as initial zooplankton availability when larval walleye first feed is important to survival (Li and Mathias 1982). Madenjian et al. (1991) used prey abundance immediately after stocking as a basis for walleye growth in a modelling exercise. Jester (1971) and Momot et al. (1977) documented poor walleye recruitment when fish prey were unavailable at the time when walleye switched to piscivory. By mediating the time it takes for saugeye to become piscivorous and thus affecting growth after the switch, variable ichthyoplankton (i.e., larval gizzard shad) densities in Ohio reservoirs (Bremigan et al. 1991; Stahl et al. 1992) could be a key factor influencing saugeye success.

Because ichthyoplankton density may affect the switch to piscivory as well as growth after the switch, we sought to determine how gizzard shad density affects saugeye growth and the time required for saugeye to switch to piscivory. We also investigated whether saugeye size at stocking affects their ability to consume gizzard shad (i.e., become piscivorous). Finally, we determined if saugeye grow faster when piscivorous, as compared with planktivorous and benthivorous. If we understand how ichthyoplankton density affects saugeye survival, then we can manage stocking time and/or saugeye size to take advantage of the abundance of fish prey to improve growth and reduce mortality.

Materials and Methods

Shad Density and the Saugeye Switch to Piscivory

We conducted all experiments at the Hebron State Fish Hatchery (Licking County, Ohio) during spring 1992. To determine if gizzard shad density influences saugeye growth and the timing of the switch to piscivory, we designed a bag experiment that consisted of two treatments, low ($20 \cdot m^{-3}$) and high ($100 \cdot m^{-3}$) gizzard shad density ($N = 8$ replicates·treatment^{-1}). Gizzard shad treatment densities were based on minimum and maximum peak ichthyoplankton densities in Ohio reservoirs stocked with YOY saugeye (Stahl et al. 1992; see Dettmers and Stein 1992 for sampling method). Experimental units were 4-mil clear plastic bags (107 × 76 × 178 cm) stapled onto wooden frames associated with a walkway constructed in a hatchery pond. To sample gizzard shad and saugeye, bags were dismantled through time. We required 64 bags for eight replicates and four dismantling dates.

On 24 May 1992, experimental bags were filled with ~1 m^3 of water, most of which was filtered through a 54-μm-mesh net. Near the end of bag filling, the filter was removed and some zooplankton was added at densities lower than high ambient pond densities, yet still greater than reservoir densities, to ensure that piscivory was not artificially induced by a deficient alternate prey source. Bags were randomly assigned treatment and dismantling dates. That night, gizzard shad larvae (20 ± 2 and 100 ± 20, low and high densities, respectively) were added to bags. Gizzard shad were 14.1 ± 0.2 mm TL (mean ± 1 SE, $N = 130$), about 40% of saugeye body length. On the following morning, six saugeye (33.9 ± 0.2 mm TL, 0.252 ± 0.005 g, $N = 63$) were added to each bag (day 0). Preliminary experiments indicated that handling mortality for gizzard shad and saugeye stocked into bags was negligible and that upon dismantling we could recover all gizzard shad added to bags (Stahl 1993).

Water temperatures inside bags were 16.5–23°C; dissolved oxygen surface to bottom ranged from 7.8 to 16.6 $mg \cdot L^{-1}$ and was measured with a YSI meter. On day 1 and immediately before the bags were dismantled, zooplankton were sampled from each bag with a clear Lexan tube sampler (7.3 cm inside diameter, 1.6 mm thick; see DeVries and Stein 1991) and preserved in 70% EtOH. One sample consisted of two 1-m water columns taken from the bag with the tube sampler and sieve through a 54-μm-mesh net. Bags were dismantled on days 2, 4, 8, and 12 to collect saugeye and gizzard shad. During dismantling, we pumped all but about 15 L of water from the bag with a hose covered by a 500-μm-mesh net to prevent fish loss. When only a few litres of water remained in the bag, we passed a hand net through the water until three successive efforts yielded no fish. Water could not be poured out of the bag due to bulkiness and the possibility of losing fish in folds. Saugeye were preserved on wet ice; gizzard shad were preserved in 95% EtOH. The experiment ended on 6 June 1992 after 12 d.

For zooplankton sample analysis, cladocerans were identified to genus and copepods were identified as calanoids, cyclopoids, or nauplii. Rotifers were not counted. For abundant taxa, a minimum of 50 individuals per sample was

counted from a thoroughly mixed subsample. For rare taxa, counting proceeded until either 50 individuals or the entire sample was counted, if the extrapolated number of individuals in each taxon was >25·sample^{-1} after one eighth of the sample was counted. If the calculated number was <25, the estimated abundance and density of that taxon were based on the number counted in the initial one eighth of the sample. Up to 22 individuals of each taxon were measured (nearest 0.001 mm) from the anterior margin of the head to the base of either the tail spine of cladocerans or the caudal rami of copepods using a digitizing board. Numbers were converted to density estimates (number per litre).

Gizzard shad from each bag were counted and measured (nearest 0.01 mm). All saugeye were measured (nearest 0.5 mm) and weighed wet (nearest 0.001 g). Before the saugeye were weighed, their stomach contents were removed, identified, counted, and measured as per the zooplankton samples. Prey length was converted individually to biomass from length – dry weight regressions (zooplankton from Dumont et al. 1975; macrobenthic organisms from G. Mittelbach, Kellogg Biological Station, Michigan State University, Hickory Corners, MI 49060, unpublished data) to estimate biomass per saugeye (grams per gram). Due to digestion, gizzard shad from guts could not be measured; their dry weights were generated from initial mean lengths. Dry weights of saugeye were obtained from the following independent regression equation: dry weight = $-0.018 + 0.21 \cdot$ wet weight ($r^2 = 0.97$, length range 25–91 mm, $N = 319$).

All bags ($N = 3$) with <4 saugeye at dismantling were not used in analyses. Those bags with more gizzard shad than stocked, within error, also were discarded ($N = 3$). These discrepancies were the result of either stocking error or bags with holes. With these exclusions, replication was 6–8 bags·treatment^{-1}·dismantling date^{-1}; for saugeye responses, the mean of 4–6 remaining saugeye per bag was a replicate in analyses. Because bags were independent through time due to dismantling, two-way ANOVAs (gizzard shad density × dismantling date) were used to analyze response variables.

Saugeye Size and the Switch to Piscivory

To judge the competency of saugeye to consume larval gizzard shad, we combined eight sizes (30–32, 32.5–33.5, 34.5–35.5, 36.5–37.5, 38.5–39.5, 41–42, 43–44, and 45–49 mm TL) of saugeye with gizzard shad (14.1 ± 0.2 mm TL). On the evening of 23 May 1992, plastic buckets (22 L, 0.28 m diameter × 0.36 m height) were filled with 10 L of hatchery-pond water filtered through 54-μm mesh. Saugeye were collected from a hatchery pond, anesthetized with quinaldine, and measured (nearest 0.5 mm). Five saugeye of a single size class were added to each bucket ($N = 3$ replicates·size class^{-1}). About 24 h later, gizzard shad were collected, counted (15 ± 2), and added by 23:00 to each bucket with 6 L more of water containing pond zooplankton. Saugeye were allowed to forage overnight and, at 10:00 the next morning, we collected the saugeye and preserved them on wet ice for stomach analysis. Gizzard shad were collected and preserved in 95% EtOH. At collection, the water temperature was 15°C.

Piscivory and Saugeye Growth

To determine if piscivory increases saugeye growth, we generated two treatments, each with four replicate ponds (0.4 ha, 0.5–1.5 m depth). One treatment contained zooplankton and chironomid larvae as prey; the other contained these prey plus larval gizzard shad.

Ponds were filled on 4 May 1992 and 60 adult gizzard shad, collected from Buckeye Lake (Licking–Fairfield counties, Ohio) on 7 May 1992, were randomly added to each of four ponds. On 21 May 1992 (day 0), once adult shad had spawned, we added about 20 000 saugeye (33.1 ± 0.2 mm TL, 0.216 ± 0.004 g, $N = 62$) to each pond. Gizzard shad were 7.69 ± 0.12 mm TL ($N = 200$) 1 d before the experiment began. Saugeye were stocked into experimental ponds in the same manner as they are stocked into reservoirs. After the rearing ponds were drained, saugeye were moved to indoor troughs, where the number in a 227-g sample was counted. All saugeye were then bulk weighed, evenly divided among eight lots by weight, trucked to each pond, and stocked.

Zooplankton and larval gizzard shad were sampled every other day starting 1 d before saugeye stocking. Zooplankton were sampled at the deep end of each pond with a tube sampler (see previous description) and preserved in 70% EtOH. One sample consisted of three 1.5-m water columns taken from the pond with the tube sampler and sieved through a 54-μm-mesh net. Larval gizzard shad were preserved in 95% EtOH after being collected by hand-hauling at the water's surface a 0.5-m-diameter, 500-μm-mesh net in open water over half the length of the pond. The net was equipped with a flowmeter mounted in its mouth to provide estimates of water volume filtered. Starting the day after saugeye were stocked, at least 10 saugeye were collected every other day before 10:00. Saugeye were collected from each pond within 1 h with a 4.6 × 1.8 m seine (3.2-mm mesh) and preserved on wet ice. All ponds were drained on 1 June 1992. Saugeye were collected, transported to indoor troughs, and numbers estimated as described previously. At least 30 saugeye from each pond were measured (nearest millimetre) and weighed (nearest 0.001 g). To compare larval gizzard shad densities between ponds with and without saugeye, on days 7 and 10 of our experiment we sampled a pond stocked with an equal number of adult gizzard shad at the same time as our experimental ponds.

We analyzed zooplankton and saugeye as described in the bag experiment, with the exception that on each sample date, ≥10 saugeye per pond were measured (nearest 0.5 mm) and weighed wet without stomach contents (nearest 0.001 g), and the stomach contents of three fish per pond were analyzed. For saugeye response variables, the mean of all saugeye analyzed per pond per sampling date was a replicate in analyses. Ichthyoplankton sample analysis consisted of counting a minimum of 200 individuals·sample^{-1} from a thoroughly mixed subsample. Fifty individuals per sample were measured (nearest 0.01 mm). Because ponds were sampled through time, repeated measures ANOVAs (gizzard shad presence × sampling date) were used to analyze response variables.

Results

Shad Density and the Saugeye Switch to Piscivory (Bag Experiment)

Whether a difference existed between original gizzard shad densities and densities on the first sampling day (day 2) was used as a qualitative metric for determining if saugeye

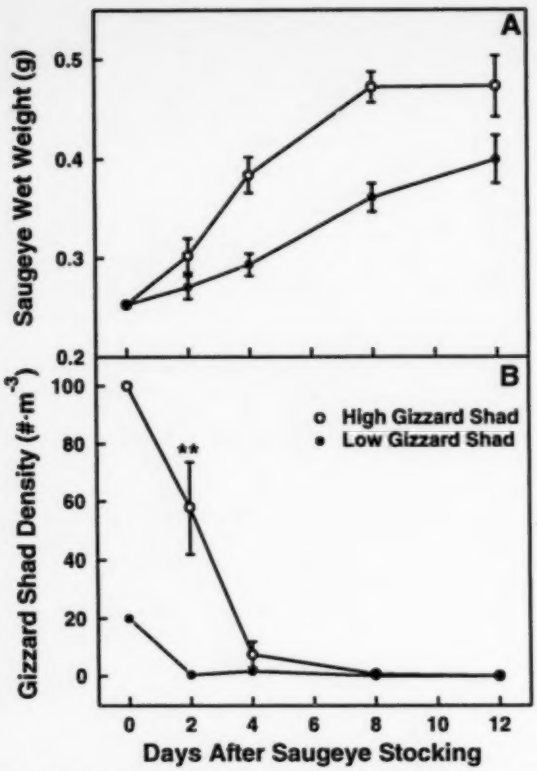

FIG. 1. (A) Mean (±1 SE) saugeye wet weight through time (N = 6–8, with 4–6 saugeye analyzed for each replicate) for the bag experiment done in 1992 at the Hebron State Fish Hatchery. Through time, saugeye wet weight increased faster in bags with high gizzard shad density (see Table 1). (B) Mean (±1 SE) gizzard shad densities through time (N = 6–8). Densities on day 0 represent gizzard shad stocked. Treatment differences (t-tests, α_{adj} = 0.0125) within a date are indicated by asterisks.

switched to fish prey in individual bags. This measure was used because recovery of gizzard shad from bags was 100%, and gizzard shad were absent from saugeye guts in most low-density treatment bags on the first sampling day. Gizzard shad were missing from bags in both treatments on day 2; in turn, frequency of bags with gizzard shad absent did not differ between treatments (Pearson chi-square, p = 0.584). Thus, treatments did not differ in switch time; saugeye were piscivorous by day 2.

Although saugeye in both densities grew (Fig. 1A) as gizzard shad densities declined (Fig. 1B), those in enclosures with 100 gizzard shad·m^{-3} grew larger than those with 20·m^{-3} (Table 1). Neither consumption point estimates of zooplankton nor chironomids (grams prey dry weight per gram saugeye dry weight) differed between treatments (Fig. 2; Table 1). Saugeye consumed gizzard shad in both treatments through day 4 (Fig. 2). However, more gizzard shad and total prey were consumed by saugeye in the high than in the low gizzard shad density treatment (Table 1). Because an interaction effect existed between treatment and dismantling day (i.e., time, see Table 1), differences in gizzard shad and total prey consumed between treatments within a single date were tested with an adjusted experimentwise α of 0.05/S (S = number of comparisons, α_{adj} = 0.0125) to determine when saugeye consumed more prey in the high gizzard shad treatment. Gizzard shad and total prey consumed were greater in the 100·m^{-3} gizzard shad treatment on day 2 (t-tests, $p < 0.001$). In the 20·m^{-3} gizzard shad treatment (Fig. 2B), saugeye diet was primarily zooplankton; few gizzard shad were consumed on days 2 and 4 because few remained in bags (Fig. 1B).

Zooplankton was abundant in both treatments. Densities in bags ranged from 800 to 2300·L^{-1} across treatments on all dates, except for bags with high gizzard shad densities on day 8, when the density was only 338·L^{-1}. Zooplankton density did not differ between treatments at initial sampling on day 1 (t-test, p = 0.87) or through time on dismantling days (Table 1). *Bosmina* spp. were the major zooplankter; treatment means for percent *Bosmina* spp. on each sample day ranged from 97.4 to 99.9%.

Gizzard shad densities in bags differed through time (Fig. 1B; Table 1). Due to an interaction effect (see Table 1), differences in gizzard shad density between treatments within a single date were also tested with an adjusted experimentwise α to determine when gizzard shad were more available in the 100·m^{-3} gizzard shad treatment. Gizzard shad densities were only greater in the 100·m^{-3} gizzard shad treatment on day 2 (t-test, p = 0.004). Densities declined dramatically through days 2 and 4 in low and high gizzard shad bags, respectively. Both low and high gizzard shad densities fell to 0·m^{-3} on days 8 and 12, respectively.

Cumulative gizzard shad consumption was positively related to change in saugeye wet weight (Fig. 3). Eliminating all data except for days after which saugeye had consumed mostly gizzard shad improved this relationship (Fig. 3).

Saugeye Size and the Switch to Piscivory (Bucket Experiment)

After 11 h of saugeye foraging, an average of 0–5.3 of the original 15 gizzard shad remained in each bucket. Number of shad consumed by different saugeye size classes did not differ (one-way ANOVA, p = 0.184). Stomach analysis of saugeye from buckets also revealed that all size classes of saugeye had consumed gizzard shad.

Piscivory and Saugeye Growth (Pond Experiment)

We ended the pond experiment on day 11 because larval gizzard shad could not be sampled with our ichthyoplankton net. Gizzard shad were not evading our net because we were able to sample them in a pond without saugeye (see Fig. 4) that had been stocked with adult shad simultaneously with our experimental ponds. No larval gizzard shad were recovered when ponds with saugeye were drained.

Saugeye grew larger in ponds with gizzard shad (Fig. 4A; Table 1) and gizzard shad densities declined concomitantly (Fig. 4B). Although saugeye survived better in ponds without gizzard shad (t-test, p = 0.021), the difference was small (97.0 versus 99.9%).

Gizzard shad did not occur in guts of sampled saugeye. Only zooplankton and macrobenthic organisms (i.e., chironomid larvae) occurred in saugeye stomachs (Fig. 5). Consumption point estimates (grams per gram dry weight) of zooplankton, chironomids, or total prey did not differ between treatments (Table 1). Because we could not explain saugeye

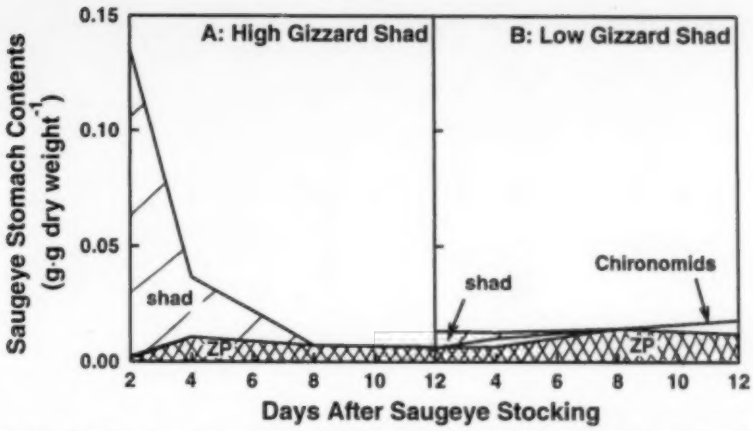

FIG. 2. Cumulative saugeye stomach contents in bags with (A) high and (B) low gizzard shad densities through time (N = 6–8, with 4–6 saugeye analyzed for each replicate) for the bag experiment done in 1992 at the Hebron State Fish Hatchery. Gizzard shad and total gut contents were greater with high gizzard shad (see Table 1); within dates, differences existed on day 2 for these measures (t-tests, α_{adj} = 0.0125). ZP = zooplankton.

TABLE 1. Significance levels (p-values) from statistical comparisons for the bag (two-way ANOVA) and pond (repeated measures ANOVA) experiments completed at the Hebron State Fish Hatchery in 1992. Response variables with units in parentheses are given for each experiment. N represents number of replicates·treatment^{-1}·dismantling/sampling day^{-1}. If saugeye were used for a response, number of saugeye·replicate^{-1} is indicated in parentheses. In sources of effects, treatment is gizzard shad availability, time is dismantling (bag) or sampling (pond) day, and interaction is between treatment and time. Significant treatment and interaction effects are indicated by an asterisk; refer to text and figures for direction of significant changes.

			Source		
Experiment	Response variable	N	Treatment	Time	Interaction
Bag	Saugeye wet weight (g)	6–8 (4–6)	<0.001*	<0.001	0.255
	Saugeye stomach contents (g·g^{-1})	6–8 (4–6)			
	Zooplankton		0.133	0.153	0.258
	Chironomid		0.062	0.217	0.109
	Gizzard shad		<0.001*	<0.001	<0.001*
	Total prey		<0.001*	<0.001	<0.001*
	Zooplankton density (no.·L^{-1})	6–8	0.915	0.021	0.353
	Gizzard shad density (no.·m^{-3})	6–8	<0.001*	<0.001	<0.001*
Pond	Saugeye wet weight (g)	4 (≥10)	<0.001*	<0.001	0.523
	Saugeye stomach contents (g·g^{-1})	4 (3)			
	Zooplankton		0.701	<0.001	0.378
	Chironomid		0.204	0.006	0.931
	Total prey		0.187	0.002	0.948
	Zooplankton density (no.·L^{-1})	4	0.025*	0.002	0.761
	Gizzard shad density (no.·m^{-3})	4	<0.001*	<0.001	<0.001*

weight differences on the basis of observed prey consumption, we examined prey availability and electivity to determine where differences existed between treatments.

Mean total zooplankton density per pond per date ranged from 467 to 14 793·L^{-1} during the experiment; densities were lower in gizzard shad ponds (Table 1). As in the bag experiment, *Bosmina* spp. were most numerous and comprised at least 84% of the zooplankton density after day 0. Calanoid, cyclopoid, nauplius, and *Daphnia* spp. densities were unaffected by gizzard shad (repeated measures ANOVA, for all treatment effects: $p > 0.05$).

Saugeye electivity (Chesson's alpha: Chesson 1978, 1983) differed among zooplankton taxa (calanoids, cyclopoids, nauplii, *Daphnia* spp., *Bosmina* spp., and other cladocerans) in both treatments. Saugeye generally preferred cyclopoids and *Daphnia* spp., showed neutral electivity for calanoids, and avoided nauplii, *Bosmina* spp., and other cladocerans. Saugeye electivity between treatments for individual taxa on each sampling day did not differ (t-tests, α_{adj} = 0.01).

Gizzard shad densities in ponds differed through time (Fig. 4B; Table 1). Due to an interaction effect (see Table 1), differences in gizzard shad density between treatments within a single date were tested with an adjusted experimentwise α of 0.05/S (S = number of comparisons, α_{adj} = 0.008) to determine when gizzard shad densities were no longer greater

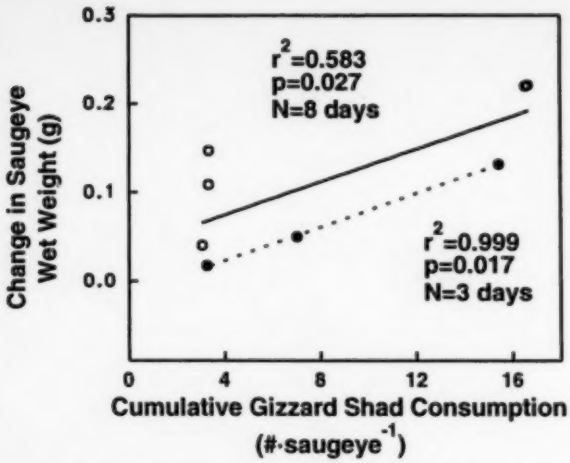

FIG. 3. Change in saugeye wet weight versus cumulative gizzard shad consumption (determined from gizzard shad bag counts) for the bag experiment done in 1992 at the Hebron State Fish Hatchery (N = 8, four dismantling dates × two treatments). The solid line is a regression for all data points; the broken line is a regression for only the solid circles. Solid circle data points represent dismantling days after which saugeye had consumed mostly gizzard shad.

in the treatment with gizzard shad. Gizzard shad densities were only greater in the gizzard shad treatment on day −1, before saugeye were stocked (Fig. 4B; t-test, p = 0.001). Gizzard shad densities declined dramatically from 71.2·m^{-3} 1 d before saugeye were stocked to <3·m^{-3} 1 d after stocking. After day 2, when densities were <1·m^{-3}, gizzard shad could still be sampled in a pond without saugeye (Fig. 4B), suggesting that gizzard shad did not evade our ichthyoplankton net.

Discussion

Factors Affecting the Switch to Piscivory

Neither saugeye size nor larval gizzard shad density affected the time required for saugeye to become piscivorous. Saugeye, as well as walleye (Parsons 1971; Knight et al. 1984; B.M. Johnson et al. 1988), can consume fish 33–50% of their total length. In our experiments, saugeye 30–49 mm consumed 14-mm gizzard shad (29–47% of saugeye TL). Also, 30-mm saugeye, smaller than those percids dealt with in other studies (34-mm walleye, Mathias and Li 1982; 39-mm saugeye, B.L. Johnson et al. 1988), were piscivorous. Although saugeye <30 mm were not tested, these fish likely can consume gizzard shad as well. YOY saugeye of the size stocked into Ohio reservoirs can consume fish.

The time for saugeye to switch to piscivory did not differ between bags containing 20 and 100 gizzard shad·m^{-3}. At both densities, saugeye consumed gizzard shad within 48 h; it is likely that the switch to gizzard shad occurred shortly after saugeye were added to bags, based on our bucket experiment results. Gizzard shad densities ≥20·m^{-3} did not reduce the time required to switch.

Although saugeye switched to piscivory within 48 h in our experiments, they do not switch this quickly when stocked into reservoirs at similar sizes (Stahl et al. 1992;

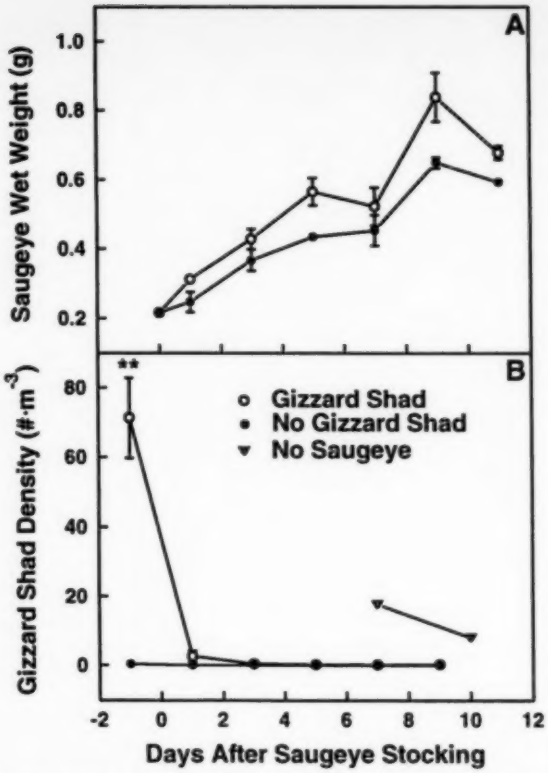

FIG. 4. (A) Mean (±1 SE) saugeye wet weight through time (N = 4 ponds, with at least 10 saugeye weighed per pond) for the pond experiment done in 1992 at the Hebron State Fish Hatchery. Through time, saugeye wet weight increased faster in ponds with gizzard shad (see Table 1). (B) Mean (±1 SE) gizzard shad densities through time (N = 4 ponds). Triangular data points are from a pond without saugeye, stocked simultaneously with the experimental ponds with an equal number of adult gizzard shad. Treatment differences (t-tests, α_{adj} = 0.008) within a date are indicated by asterisks.

Stahl 1993). Conceivably, larval gizzard shad densities <20·m^{-3} could inhibit the switch to piscivory by either being below an optimal gizzard shad density in relation to other prey or falling below a density where saugeye learn to consume a novel prey item. In our experiments, saugeye drove gizzard shad densities to zero; thus, this predator can exploit low prey densities and would likely switch to piscivory at low gizzard shad densities. However, high gizzard shad densities may be required for saugeye to switch to piscivory initially; once a single gizzard shad is captured, then saugeye may continue to consume shad. Saugeye exposed to low densities initially will experience a reduced encounter rate, which might deter pursuit because of added search time. Coupling low densities of gizzard shad with shorter dismantling times in another bag experiment would provide insight into required threshold densities for the switch to fish prey.

One factor other than ichthyoplankton density that may cause a delayed switch to piscivory by saugeye is an abundant, alternate preferred prey. Large zooplankton have been mentioned as important for walleye growth and survival (Fox et al. 1989; Fielder 1992) and might be sufficiently

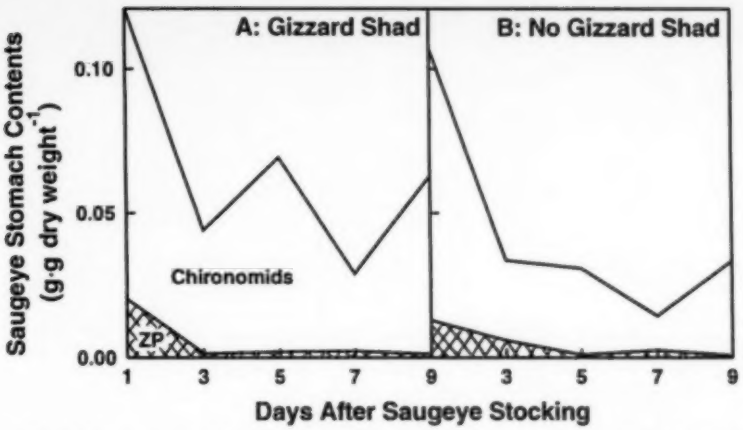

FIG. 5. Cumulative saugeye stomach contents in ponds (A) with and (B) without gizzard shad through time (N = 4, with at least 3 saugeye analyzed per replicate) for the pond experiment done in 1992 at the Hebron State Fish Hatchery. Zooplankton, chironomid, and total stomach contents did not differ (see Table 1). ZP = zooplankton.

abundant in reservoirs to reduce the attractiveness of gizzard shad, thus delaying the switch to piscivory. Although large zooplankton (cyclopoids and *Daphnia* spp.) were preferred in our experiments, these taxa comprised <<10% of the community. However, when the combined absolute densities of cyclopoids and *Daphnia* spp. in the ponds (19–125·L^{-1}·$pond^{-1}$·$date^{-1}$), and to a lesser extent in the bags (0–37·L^{-1}·$treatment^{-1}$·$date^{-1}$), and to a lesser extent in the bags (0–37·L^{-1}·$treatment^{-1}$·$date^{-1}$), are compared with maximum densities of cyclopoids and *Daphnia* spp. in four Ohio reservoirs sampled in 1991 (10–56·L^{-1}·$reservoir^{-1}$; Stahl et al. 1992), preferred zooplankton abundance did not appear to be limiting in our experiments It is likely not the factor inhibiting the switch to piscivory by saugeye in Ohio reservoirs. A delayed switch also could result from spatial separation of saugeye and larval gizzard shad. YOY saugeye are collected inshore with seines and electrofishing gear (B.L. Johnson et al. 1988; Stahl et al. 1992), but their distribution within reservoirs as a whole is unknown. Although larval gizzard shad are present inshore and offshore at the water's surface (Petering and Johnson 1991; Allen and DeVries 1993), and this fish is the primary prey for stocked saugeye (B.L. Johnson et al. 1988; Stahl et al. 1992), the extent to which these fishes overlap spatially in the water column is unknown in Ohio reservoirs. B.L. Johnson et al. (1988) indicated that saugeye do consume more littoral fishes than walleye, indicating a possible spatial separation from larval gizzard shad found primarily in the open water. Just as larval fish increase activity and range with size (Miller et al. 1988), so may saugeye and gizzard shad. Thus, the switch to piscivory may be delayed by inadequate spatial overlap, being resolved when saugeye or gizzard shad increase their range through growth, as shown for walleye and yellow perch (*Perca flavescens*) (Lyons 1987). Quantifying the spatial distribution of YOY saugeye and gizzard shad would provide insight into this issue. Finally, a perceived delayed switch could derive from sampling bias, as has been documented for fish larvae and their zooplankton prey (Frank 1988). If spatial distribution or range of YOY saugeye increases with size, and small saugeye remain inshore, and thus do not overlap with ichthyoplankton and consume zooplankton, then inshore sampling biases our view of the time required for saugeye to switch to piscivory. To determine the presence, size distribution, and diets of saugeye offshore, trawling or other methods should be attempted.

Piscivory and Growth

Although no gizzard shad were found in stomachs of pond saugeye, saugeye probably consumed these shad. First, increased growth in the treatment with gizzard shad was not caused by differences in prey found in saugeye stomachs. Weight of food in saugeye guts did not differ with and without gizzard shad. Saugeye growth differences were likely derived from differences in prey consumption that we were unable to quantify, specifically gizzard shad consumption. Second, gizzard shad availability was the only difference in prey between treatments that could have increased saugeye growth in the treatment with gizzard shad. Although zooplankton densities were higher through time without gizzard shad, saugeye still grew fastest with gizzard shad. Densities of preferred zooplankters (cyclopoids and *Daphnia* spp.) and zooplankton electivity did not differ between treatments. Finally, saugeye had the ability to consume all gizzard shad and clear their guts by the time we first sampled 24 h after stocking. Calculations, based on YOY walleye bioenergetics (Madon and Culver 1993), show that saugeye possess the predatory and consumption potential (0.30 g·g^{-1}·d^{-1} for comparable YOY walleye, Culver et al. 1992) necessary to eliminate gizzard shad from the pond and their stomachs within 24 h (Stahl 1993). Walker and Applegate (1976) also documented the elimination of forage fish within 8 wk by stocked walleye (N = 25 000, 43.5 mm) in a prairie pothole. Given this tremendous predatory potential, it is not surprising that no gizzard shad were recovered from guts. Although no formal saugeye-free control ponds existed, we believe that gizzard shad were not evading our gear because we were able to sample them in a predator-free pond that had been stocked with the same number of adult gizzard shad simultaneously with our experimental ponds. Gizzard shad in our ponds with saugeye were likely eliminated by saugeye.

Piscivorous saugeye grew faster than zooplanktivorous/benthivorous ones, as evidenced by both pond and bag experiments. Gizzard shad consumption drove saugeye weight gain. The influence of gizzard shad on growth was impressive — even though saugeye in ponds did not have access to gizzard shad for 10 days and grams per gram consumption was similar following gizzard shad elimination, their consumption of shad set the trajectory of the growth curve early, permitting saugeye in gizzard shad ponds to outgrow those in ponds without gizzard shad. In our bag experiment, saugeye that had access to more gizzard shad grew faster. On days 0 and 2, respectively, high-density bags had 100 and 58 gizzard shad, as compared with 20 and <1 gizzard shad in low-density bags. Thus, saugeye in the $100 \cdot m^{-3}$ bags grew larger. As in the pond experiment, gizzard shad consumption set the trajectory of the growth curve early; saugeye in the high gizzard shad treatment were larger through the entire experiment despite gizzard shad availability and consumption being greater only through day 2. Further, by using only the bag treatment data after which gizzard shad had been consumed, the number of gizzard shad consumed by saugeye precisely predicted saugeye growth.

Several mechanisms could account for greater growth of saugeye consuming gizzard shad as compared with those consuming zooplankton or chironomids. First, caloric densities of larval fish exceed those of chironomids or zooplankton (Cummins and Wuycheck 1971; Hewett and Johnson 1992). In addition, assimilation efficiency for fish prey is greater than that for invertebrates (Brett and Groves 1979). Therefore, weight gain by a predator from an equal biomass of each prey item should be greatest for YOY fish. Also, because saugeye are natural piscivores, they may be able to forage for fish more efficiently than for either chironomids or zooplankton. For example, on day 2 of the bag experiment, greater total biomass of prey in saugeye stomachs in the high gizzard shad treatment coincided with greater gizzard shad consumption. Greater efficiency in assimilation and consumption coupled with high caloric density of fish likely accounted for faster saugeye growth in ponds, as well as bags, with gizzard shad.

Although saugeye survival was higher in ponds without gizzard shad, differences were small (<3%) and likely more related to our counting procedures than gizzard shad presence. In fact, in productive hatchery ponds, differences in survival based on prey availability would be unexpected over only 11 d (Fox 1989).

Our experiments did not determine if increased ichthyoplankton density increases saugeye growth. Clearly, when saugeye co-occurred with gizzard shad, they grew faster. However, we did not maintain constant gizzard shad densities in our experiments; therefore, these data do not provide insight into the prey density required for maximum saugeye growth. As with most animals, saugeye consumption and growth will increase with increasing prey abundance until a maximum is reached. Understanding how maximum saugeye growth is achieved given different saugeye stocking parameters (size, density, and time) and larval gizzard shad population dynamics would be useful to managers. Greater understanding can be achieved through manipulative saugeye growth experiments, field sampling of YOY saugeye and ichthyoplankton, bioenergetics models specific for 30- to 50-mm saugeye (sensu B.M. Johnson et al. 1988; Wahl and Stein 1991; Madon and Culver 1993), and, given the importance of size for YOY predators (Gutreuter and Anderson 1985; Miller et al. 1988; Madenjian and Carpenter 1991; Madenjian et al. 1991), an individual-based model for saugeye (sensu Madenjian and Carpenter 1991; Madenjian et al. 1991).

Management Recommendations

In nature, production of larval fish predators often coincides with production of abundant prey fish, which in turn enhances predator growth and survival (Momot et al. 1977; Adams and DeAngelis 1987; Wicker and Johnson 1987). Timing is important for stocked predators simply because prey fish must be appropriately sized (B.M. Johnson et al. 1988) at a density that can sustain high growth rates (Jester 1971; Stahl et al. 1992). Managers can control these factors by manipulating saugeye size, reservoir type, or the time of stocking in relation to prey density or size. Our experiments demonstrate that saugeye as small as 30 mm could consume gizzard shad at densities of $\geq 20 \cdot m^{-3}$, and through this consumption increase their growth. Thus, we conclude that saugeye should be stocked as large as possible, but at least 30 mm, 1–2 wk before peak ichthyoplankton densities, in reservoirs where gizzard shad occur.

Stocking at this size and time is advantageous for a variety of reasons. First, with this scenario, saugeye capable of consuming larval gizzard shad will have energetically favorable fish prey maximally available soon after stocking. Second, stocking saugeye slightly before the peak will allow saugeye time to increase their range to overlap spatially with, and perhaps "learn" to consume, prey fishes. Thus, any delay in switching to piscivory upon stocking occurs before peak ichthyoplankton densities, preventing saugeye from missing abundant prey, which can grow quickly out of their window of vulnerability (B.M. Johnson et al. 1988). Likewise, fast growth by gizzard shad makes stocking saugeye larger than 30–50 mm questionable. If reared in hatchery ponds, saugeye >50 mm may not be available for stocking until YOY gizzard shad are beyond a size range vulnerable to saugeye. Finally, although peak ichthyoplankton densities cannot be precisely predicted, they occur during 2 wk in mid-May to early June in Ohio reservoirs (Stahl et al. 1992, 1993). Until methods for predicting gizzard shad hatch dates and recruitment to the pelagia have been developed, using historical data to predict peak ichthyoplankton densities should suffice. Without these data, timing of peaks can be bracketed by sampling ichthyoplankton at least weekly in spring for several years. By stocking ≥30-mm saugeye 1–2 wk prior to ichthyoplankton peaks, we believe that saugeye will be able to take advantage of larval gizzard shad, thereby improving their growth, survival, and ultimate recruitment to the fishery.

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Abiotic and Seasonal Factors Influencing Trace Metal Levels (Cd, Cu, Ni, Pb, and Zn) in the Freshwater Amphipod *Gammarus fasciatus* in Two Fluvial Lakes of the St. Lawrence River

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Amyot, M., B. Pinel-Alloul, and P.G.C. Campbell. 1994. Abiotic and seasonal factors influencing trace metal levels (Cd, Cu, Ni, Pb, and Zn) in the freshwater amphipod *Gammarus fasciatus* in two fluvial lakes of the St. Lawrence River. Can. J. Fish. Aquat. Sci. 51: 2003–2016.

The potential use of the freshwater amphipod *Gammarus fasciatus* as a biomonitor of metal pollution has been assessed in two fluvial lakes of the St. Lawrence River. Metal (Cd, Cu, Fe, Mn, Ni, Pb, and Zn) concentrations were measured twice at 22 sites in Lakes St. Pierre and St. Louis, both in oxic sediments and in amphipods. Concentrations of free-metal ions at the sediment–water interface were estimated from lake water pH and sediment/water sorption equilibria. Multiple linear regression models forecasting trace metal (Cd, Cu, Ni, Pb, and Zn) levels in amphipods were developed, using abiotic variables (sediment-bound metals, sediment organic carbon, free-metal ions, hydrogen ions, dissolved calcium, and dissolved organic carbon) and biotic (size class) variables. These models explained 73–81% of the observed variation, except in the case of Zn (68%) and Cu (61%), indicating that *G. fasciatus* is a promising biomonitor for Cd, Ni, and Pb in littoral environments. Seasonal patterns of amphipod contamination were identified for Fe, Ni, and Pb, summer concentrations being higher than those measured during the fall. Size-dependent variation in amphipod metal levels was found, smaller organisms being more contaminated than larger ones.

On a évalué la possibilité d'utiliser l'amphipode d'eau douce *Gammarus fasciatus* comme bioindicateur de la pollution par les métaux dans deux lacs fluviaux du fleuve Saint-Laurent. Les concentrations de métaux (Cd, Cu, Fe, Mn, Ni, Pb et Zn) ont été mesurées deux fois à 22 endroits dans les lacs Saint-Pierre et Saint-Louis, dans les sédiments oxiques et chez les amphipodes. Les concentrations d'ions métalliques libres à l'interface sédiment–eau ont été estimées à partir du pH de l'eau du lac et des données de sorption à l'équilibre à l'interface sédiment–eau. On a mis au point des modèles de régression linéaire multiple pouvant prédire les concentrations de métaux à l'état de traces (Cd, Cu, Ni, Pb et Zn) chez les amphipodes, à l'aide de variables abiotiques (métaux liés aux sédiments, carbone organique des sédiments, ions métalliques libres, ions hydrogène, calcium dissous et carbone organique dissous) et de variables biotiques (catégories de taille). Ces modèles expliquaient 73 à 81 % des variations observées, sauf dans le cas du Zn (68 %) et du Cu (61 %), ce qui indique que *G. fasciatus* est un bioindicateur prometteur pour le Cd, le Ni et le Pb dans les environnements littoraux. On a déterminé des variations saisonnières de contamination des amphipodes par le Fe, le Ni et le Pb, les concentrations étant plus élevées en été qu'à l'automne. On a observée des variations de la concentration des métaux en fonction de la taille des amphipodes, les petits organismes étant plus contaminés que les gros.

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During the last century, increased industrialization has led to important point-source discharges of metals to freshwater ecosystems. Human activities have also caused an increase in atmospheric deposition of metals, resulting in environmental contamination problems even in remote lakes. In many ecosystems, these human-induced changes have overwhelmed the natural biogeochemical fluxes of trace elements (Nriagu 1990).

Metals entering freshwater environments partition among various compartments (water, suspended particulate matter, sediments, and biota). In particular, freshwater sediment acts as an important reservoir for metals, with concentrations attaining levels 1000–5000 times higher than those in the overlying water column (Campbell and Tessier 1991). This metal contamination of surficial sediments is of ecotoxicological importance, in particular for benthic animals and plants.

This study focuses on the potential use of the macrophyte-associated amphipod *Gammarus fasciatus* as a biomonitor for the sediment contamination of Cd, Cu, Ni, Pb, and Zn in two areas of seasonal sedimentation along the St. Lawrence River (Lakes St. Pierre and St. Louis). These metals were chosen because of their relatively high toxicity and bioavailability (Campbell et al. 1988) and their elevated concentrations

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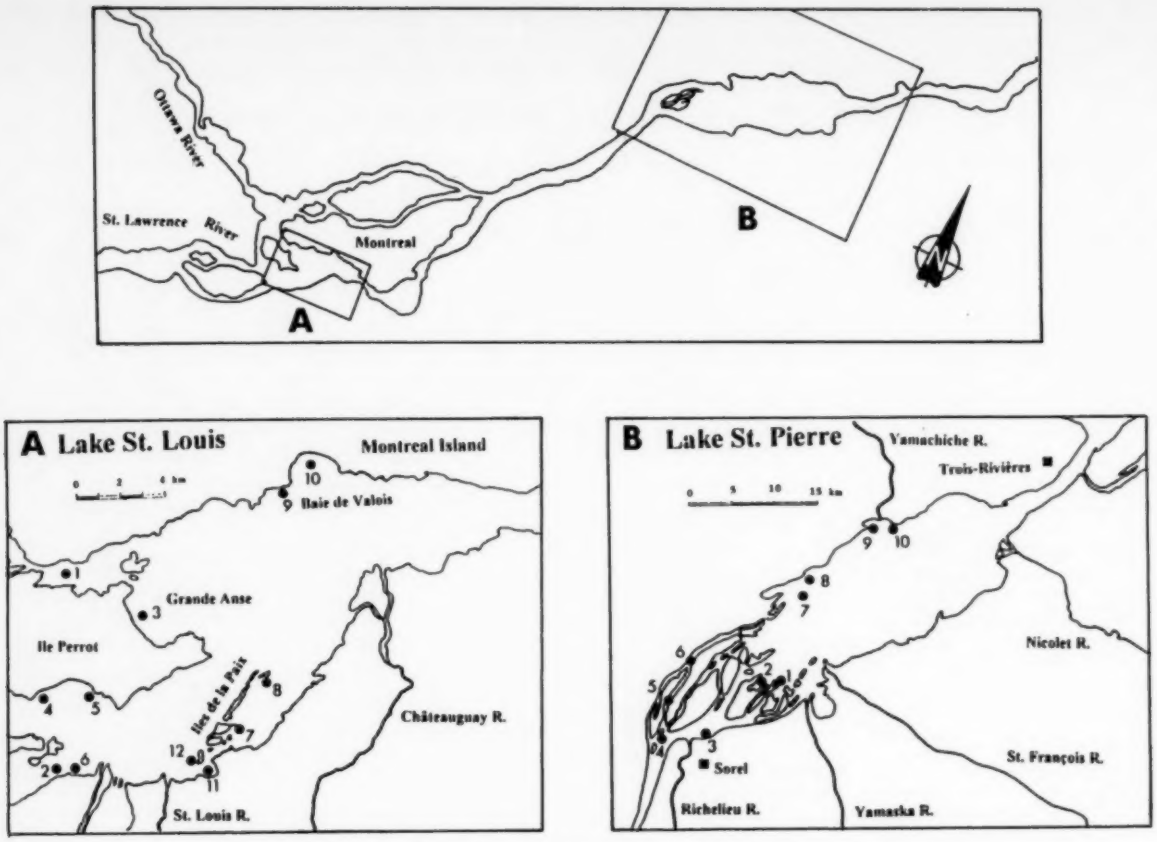

FIG. 1 Location of sampling sites in (A) Lake St. Louis and (B) Lake St. Pierre.

in the studied areas (Champoux and Sloterdijk 1988). Furthermore, Mn and Fe have also been included in the analysis, since these metals are intimately involved in sorption processes affecting the bioavailability of other sediment-bound metals (Tessier 1992).

The choice of the St. Lawrence River as a study site is particularly relevant, since the Canadian government has recently implemented a program aimed at eliminating 90% of the toxic discharges from the 50 most polluting industries on the shoreline. Thus, there is a need for reliable biomonitoring tools for this area.

Among the organisms encountered in the St. Lawrence River ecosystem, the amphipod *G. fasciatus* seems, a priori, to have many of the properties of the ideal biomonitor, as identified by Rainbow et al. (1989). This species is easily recognizable, common, and accessible (Dumouchel 1969) and survives handling for complementary experiments (Arthur 1980). Other studies in marine systems have already shown the value of some amphipods as useful biomonitors (Rainbow et al. 1989; Moore et al. 1991). Furthermore, *G. fasciatus* is part of the macrophyte-associated community, feeding on sediments, macrophytes, and periphyton (Clemens 1950). This community has rarely been studied on an ecotoxicological basis, even though it plays a major role in the trophic transfer of energy and reduced carbon (Boisclair and Leggett 1989) and, accordingly, may be of considerable importance in pollutant transfer.

Little is known about routes of metal uptake by *G. fasciatus*. As a first step in the evaluation of the usefulness of this amphipod as a biomonitor, this study was designed to (1) determine whether *G. fasciatus* can be used as a biomonitor of the degree to which sediments are contaminated with certain metals, (2) evaluate the effect of body size on metal accumulation, and (3) consider the importance of seasonal variations in amphipod metal concentrations. In the aqueous phase, selected factors included the concentrations of hydrogen ions, dissolved calcium, dissolved organic carbon (DOC), and free-metal ions. In the sediments, the factors considered were total organic carbon, sediment-bound metals, and iron and manganese oxides. The predictive power of these various factors was investigated with multiple linear regression models.

Materials and Methods

Study Sites

This study was carried out in Lakes St. Pierre and St. Louis, both of which are widenings of the St. Lawrence River, one midway between Montreal and Quebec City and the other south of the island of Montreal (Fig. 1).

Lake St. Pierre, the largest of the St. Lawrence fluvial lakes, is located downstream from Sorel (Quebec, Canada). Inputs to the lake include the St. Lawrence River itself and a number of major tributaries draining northward from the

Appalachians (Richelieu, Yamaska, St-François, and Nicolet rivers). The upstream part of the lake is characterized by the presence of an intricate network of channels and small islands, which would be expected to enhance the sedimentation rates of pollutants. Major pollution sources for the littoral region of the lake are tributaries downstream from Sorel (Richelieu, St-François, and Yamaska rivers). Also of major concern are sewage outfalls from local industries and municipalities in the Sorel area. A more detailed description of this water body can be found in Sylvestre et al. (1992).

Ten sampling stations were chosen in littoral macrophyte beds in Lake St. Pierre, six of them being located in the Sorel Islands (stations 1–6) and the others being downstream, on the north shore of the lake (stations 7–10) (Fig. 1). Site visits were carried out in August and October 1990.

The other sampling locale was Lake St. Louis (Fig. 1), the reach of the St. Lawrence River lying to the south of Montreal (Quebec, Canada). It is considered to be one of the most polluted water bodies in Quebec. The dominant input to the lake is the St. Lawrence River itself, the waters derived from the Laurential Great Lakes being highly mineralized and known locally as "green". The second important tributary is the Ottawa River, which is characterized by dilute brown waters. Whereas the local pollution sources affecting Lake St. Pierre are concentrated near Sorel, those influencing Lake St. Louis are more scattered. In the latter, there are three main areas where sediment metal concentrations are high: (1) midway between "Baie de Valois" and "Grande Anse", (2) south of "Îles de la Paix", near the mouth of the St. Louis River, and (3) south of Île Perrot", near the mouth of the Ottawa River. The main sources of point discharges are located in the industrial complex near Beauharnois and along the St. Louis River. A more detailed description of these areas and further information about this lake are available in Jarry et al. (1985).

Twelve stations were chosen in littoral macrophyte beds in Lake St. Louis, some of these being in the brown waters dominated by inputs from the Ottawa River (stations 1, 4, and 5) or from the St. Louis River (stations 7, 8, 11, and 12) and others being in the green waters of the St. Lawrence River (stations 2, 3, and 6) (Fig. 1). Sites were visited in July and August 1991.

Trace Metal Distribution in Sediments

Sediments were sampled at each station by SCUBA divers using polypropylene core samplers. The uppermost 0.5 cm, containing oxidized sediments, was then removed with a Teflon sheet and transferred to an acid-cleaned polypropylene centrifugation bottle. To reduce the effect of local (within-site) spatial variability, each bottle contained four to six slices of oxidized sediments, each slice coming from a different core. The cores were collected randomly in a 100-m^2 area. One bottle of pooled sediment was obtained for each site. In addition, four replicates were processed at stations 4 and 9 of Lake St. Pierre and at stations 3, 4, 6, and 8 of Lake St. Louis. The mean coefficient of variation (CV) for levels of potentially available sediment-bound metals ($\{M_s\}$) among replicate samples collected at a given site was 12%. The bottles were then frozen at −20°C until further processing in the laboratory.

Thawed sediments were analyzed using a sequential extraction procedure developed by Tessier et al. (1979). This method is designed to partition particulate trace metals into the following operationally defined fractions: (1) exchangeable metals: the sediment was extracted for 30 min with 0.5 M $MgCl_2$ at pH 7.0, (2) metals bound to carbonates or specifically adsorbed: the residue from (1) was leached for 5 h with 1 M sodium acetate (NaOAc) adjusted to pH 5.0 with acetic acid (HOAc), and (3) metals bound to iron and manganese oxides: the residue from (2) was extracted at 96°C for 6 h with 0.04 M $NH_2OH{\cdot}HCl$ in 25% (v/v) HOAc.

Metal concentrations in the leachates were determined by flame or graphite tube atomic absorption spectrophotometry and by inductively coupled plasma atomic emission spectroscopy. To evaluate the total concentration of potentially available sediment-bound metals ($\{M_s\}$), metal concentrations in the first three fractions were summed.

The content in organic matter of sediments was determined by loss on ignition. These values were converted into concentrations of organic carbon using the Van Bemmelen factor: organic matter (%) = organic carbon (%) × 1.724 (Kalra and Maynard 1991).

TABLE 1. Values used in the calculation of the free-metal ion concentration. Values for Cu, Ni, Pb, and Zn are from Tessier (1992) and those for Cd from Tessier et al. (1993).

Metal	$N_{Fe}{\cdot}K_{Fe\text{-}M}$	$N_{MO}{\cdot}K_{MO\text{-}M}$	x	y
Cu	1.26	—	0.64	—
Ni	0.00512	—	1.04	—
Pb	4.68	—	0.81	—
Zn	0.00148	—	1.21	—
Cd	0.05012	0.00355	0.82	0.97

Free-Metal Ion Concentrations

Free-metal ion concentrations (molar) were estimated with equations given by Tessier (Tessier 1992; Tessier et al. 1993). This approach assumes that the free-metal ion concentration at the sediment–water interface is controlled by competitive sorption of the metal on the various sediment phases present in oxic sediments. For the metals Cu, Ni, Pb, and Zn, we assume that iron oxyhydroxide surfaces play an important role in this adsorptive control of the free-metal ion concentration (Tessier 1992) and that the amount of metal sorbed to this phase can be approximated by $\{M_s\}$.

Surface complexation concepts (Benjamin and Leckie 1991) can be used to describe this sorption (Eq. 1):

$$(1)\quad \equiv FeOH_x + M^{2+} \overset{K_a}{\leftrightarrow} \equiv Fe\text{–}OM + xH^+$$

and the free-ion concentration can be expressed as

$$(2)\quad [M^{2+}] = \frac{\{M_s\}[H^+]^x}{N_{Fe} \cdot K_{Fe\text{-}M}}\{Fe_s\}$$

where $\{M_s\}$ is the total concentration (moles per gram) of metal sorbed to the iron oxyhydroxides, $[H^+]$ is the hydrogen ion concentration (molar) in the water column, x is the average apparent number of protons released per metal ion sorbed, N_{Fe} is the number of moles of adsorption sites on the iron oxyhydroxides per mole of Fe, $K_{Fe\text{-}M}$ is an apparent overall equilibrium constant, and $\{Fe_s\}$ is the analytical concentration of iron oxyhydroxides (moles Fe per gram). Throughout this paper, the notation "≡" refers to adsorption sites whereas { } and [ ] refer to concentrations of solid

and dissolved species, respectively. Equation 2 was used to evaluate the free-metal ion concentrations of Cu, Ni, Pb, and Zn. Values of the product $N_{Fe} \cdot K_{Fe-M}$ were taken from Tessier (1992) (Table 1).

For Cd, sorption of metal ions on organic matter was also considered, since additional empirical constants were available from Tessier et al. (1993). Free-Cd concentrations (molar) were expressed as

$$(3) \quad [Cd^{2+}] = \frac{\{Cd_s\}[H^+]^{x+y}}{N_{Fe} \cdot K_{Fe-Cd}\{Fe_s\}[H^+]^y + N_{MO} \cdot K_{MO-Cd}\{C_{org}\}[H^+]^x}$$

where $\{Cd_s\}$ is the total sorbed Cd concentration (moles per gram), y is the apparent number of protons released per Cd^{2+} sorbed on organic matter, N_{MO} is the number of moles of sites on the organic matter per mole organic carbon, K_{MO-Cd} is an apparent equilibrium constant, and $\{C_{org}\}$ is the concentration or organic carbon (moles per gram). Values of $N_{Fe} \cdot K_{Fe-Cd}$ and $N_{MO} \cdot K_{MO-Cd}$ were taken from Tessier et al. (1993 (Table 1).

Water Analysis

Measurements of dissolved oxygen (oxymeter YSI-57), pH (pH-meter Fisher Accumet 620), and conductivity (conductivimeter Cole Parmer 1484-10) were taken in the field at a depth of 0.5 m. Samples for dissolved Ca concentrations were collected at the same depth and acidified with nitric acid (0.5%). Ca was analyzed by flame atomic absorption spectrophotometry using a lanthanum–cesium suppressor.

Metal Concentrations in Amphipods

Amphipods were sampled in macrophyte beds by SCUBA divers using nets. In the laboratory, animals were divided into three size classes (estimated body length of living animals: 3–7, 7–9, and 9–12 mm). They were then put on blotting paper (KimWipes™) to remove excess water. Undepurated organisms of the same size class were pooled in a polypropylene bottle. Approximately 100 mg of equivalent dry weight was put in each vial. One to three replicates for each size class were prepared, depending on the abundance of organisms in each size class. The bottles were stored in a freezer at −20°C. All sampling bottles and laboratory glassware were initially acid washed with nitric acid (15%).

Metal analysis in amphipods was based on a method described in Hare et al. (1991b). Samples were dried for 18 h at 70°C and placed in Teflon bombs with ultrapure nitric acid (Aristar grade) in an approximate ratio of 3 mL of nitric acid per 100 mg dry weight. The bombs were heated in a microwave oven at 360 W for 1.5 min. Digestion solutions were diluted with Millipore water to obtain a 12% nitric acid concentration. To monitor analytical performance, samples of similar weight of two certified reference materials (lobster hepatopancreas tissue, NRCC Tort; oyster tissue, NIST SRM 1566) were digested on a regular basis; measured trace metal concentrations in the standards varied little over time (Tort: CV = 5–17%; oyster tissue: CV = 2–14%) and were within the certified ranges for each element. Blanks were also run and no appreciable contamination was detected. Metal concentrations were determined by flame and graphite tube atomic absorption spectrophotometry.

Statistical Analyses

For all parametric analyses, metal concentrations in amphipods were normalized using a logarithmic transformation ($\log_{10}$).

To assess whether amphipod contamination varied among organisms of different sizes or among different sites, Friedman's two-way analysis of variance was used (Sokal and Rohlf 1981). A first test was computed where size classes were considered as factors and sites were considered as blocks. A second test was made where sites were considered as factors and size classes as blocks.

Seasonal changes in metal levels in amphipods were assessed for Lake St. Pierre, where sampling was carried out in summer (August 1990) and fall (October 1990). A t-test for paired comparisons was used, samples of each size class from each site for a given season being paired with the corresponding sample from the other season. A similar analysis was conducted to evalute within-season changes in amphipod metal concentrations, results from July 1991 being compared with those of August 1991 (Lake St. Louis) using the pairing system. Since 14 t-tests were computed, each of them was done at $\alpha/14$ level of significance, using the Bonferroni correction (Kirk 1982).

Empirical multiple linear regression models were constructed in order to examine the extent to which observed trace metal levels in amphipods could be explained by a limited set of abiotic variables. To eliminate the influence of seasonal factors, only data from August 1990 (Lake St. Pierre) and August 1991 (Lake St. Louis) were considered. To determine whether samples collected at sites within the same lake could legitimately be considered as independent, a cluster analysis was performed using the log-normalized values of abiotic variables related to water and sediment chemistry at each site ($[H^+]$, $[Ca^{2+}]$, $\{Fe_s\} + \{Mn_s\}$, $\{C_{org}\}$, and DOC). To decrease the risk of spatial autocorrelation, pairs of stations exhibiting all the following characteristics were selected: (1) stations less than 5 km apart, (2) stations clustered together, and (3) stations separated by less than 0.2 unit in euclidean distance. One station of each selected pair was discarded from the multiple regression.

Based on the available literature, several potentially powerful predictors were chosen for incorporation in the regression models. The abiotic factors were related to both the sediment and the water phases and included hydrogen ion concentration ($[H^+]$), which may affect metal speciation in solution or biological sensitivity at the level of the biological surface (Campbell and Stokes 1985), the concentration of dissolved Ca ($[Ca^{2+}]$), Ca^{2+} being believed to compete with other divalent metals at fixation sites on the cell membrane (Stephenson and Mackie 1988, 1989), the concentration of DOC, DOC being known to complex trace metal ions, largely in the humic and fulvic acid fractions (Neubecker and Allen 1983), the calculated free-metal ion concentrations ($[M^{z+}]$), these ions being considered as the best predictors of metal bioavailability, according to the free-ion activity model (Morel 1983), the concentration of relatively available sediment-bound metals ($\{M_s\}$), these metals being presumed to be in sorptive equilibrium with $[M^{z+}]$ (Tessier 1992), and the sediment concentration of organic carbon ($\{C_{org}\}$) and of iron and manganese oxides ($\{Fe_s\}$) + ($\{Mn_s\}$), these variables having been shown empirically to influence sediment metal bioavailability (Tessier and Campbell 1990). Amphipod size classes were represented by dummy variables (Neter et al. 1990).

TABLE 2. Mean concentrations of potentially available sediment-bound metals ($\{M_s\}$), sediment organic carbon ($\{C_{org}\}$), water pH, dissolved Ca, and dissolved organic carbon (DOC) for each site and each sampling period: Lakes St. Pierre and St. Louis.[a]

Lake St. Pierre sites	$\{Mn_s\}$ ($\mu mol \cdot g^{-1}$)		$\{Fe_s\}$ ($\mu mol \cdot g^{-1}$)		$\{Pb_s\}$ ($nmol \cdot g^{-1}$)		$\{Ni_s\}$ ($nmol \cdot g^{-1}$)		$\{Cu_s\}$ ($nmol \cdot g^{-1}$)		$\{Zn_s\}$ ($nmol \cdot g^{-1}$)		$\{Cd_s\}$ ($nmol \cdot g^{-1}$)		$\{C_{org}\}$ ($mmol \cdot g^{-1}$)		pH		[Ca] ($\mu mol \cdot L^{-1}$)		DOC ($mg \cdot L^{-1}$)	
	A90	O90	A90	O90	A90	O90	A90	O90	A90	O90	A90	O90	A90	O90	A90	O90	A90	O90	A90	O90	A90	O90
1	5.33	5.20	97.2	110	60	82	146	153	12	24	948	1560	4.89	5.25	2.54	2.74	8.22	7.34	661	980	2.6	2.7
2	3.71	7.88	112	131	78	79	153	186	56	61	1220	1600	6.23	7.21	2.46	2.69	8.13	7.30	694	623	2.5	2.9
3	1.65	1.31	49.2	42.6	49	49	76	81	35	40	298	257	0.80	0.27	0.46	0.32	7.59	7.20	654	781	2.7	4.1
4	3.89	3.87	116	87.9	94	59	130	124	88	65	1300	1070	5.43	2.83	2.13	1.40	7.36	6.82	447	396	3.6	5.5
5	8.15	7.50	155	165	107	106	212	242	120	137	1770	2390	8.45	9.43	3.83	3.36	7.14	7.07	427	309	3.9	5.6
6	2.87	6.84	86.1	140	39	51	97	155	38	81	750	1370	3.47	4.27	2.32	3.05	6.89	7.02	422	479	4.2	5.9
7	1.35	3.44	45.6	71.4	29	41	70	98	47	48	384	769	1.69	0.62	0.55	1.08	6.77	7.10	487	417	3.8	1.2
8	4.07	3.71	76.5	78.8	48	72	96	118	63	75	773	1050	2.58	4.36	1.62	1.35	8.50	7.11	454	342	4.1	5.3
9	4.15	4.09	89.8	117	20	10	60	69	50	46	516	828	1.07	0.36	1.04	1.13	8.36	7.03	387	294	3.8	—
10	1.15	1.72	35.6	47.3	1.4	1.5	27	23	20	29	222	389	0.27	0.27	0.18	0.19	7.77	6.84	247	247	3.8	6.1
Max:min	7.1	6.0	4.4	3.9	76	71	7.9	11	10	5.7	8.0	9.3	31	35	21	18	54[b]	3.3[b]	2.8	4.0	1.7	5.1

Lake St. Louis sites	J91	A91	J91	A91	J91	A91	J91	A91	J91	A91	J91	A91	J91	A91	J91	A91	J91	A91	J91	A91	J91	A91
1	24.2	13.7	186	167	112	114	197	161	28	32	1867	1601	7.5	7.1	3.16	3.41	7.00	6.91	185	198	5.3	5.7
2	1.83	3.78	29.2	54.6	18	43	22	74	7.4	5.1	875	1945	4.1	12.4	1.08	1.92	7.66	8.08	872	830	2.0	2.5
3	3.23	0.30	54.8	11.8	39	5	63	9	22	6.4	827	102	2.8	0.5	0.67	0.17	8.20	8.40	793	802	2.9	2.4
4	15.1	18.9	147	163	18	13	248	214	189	132	512	519	0.8	1.0	0.92	0.75	8.15	7.26	677	309	3.2	5.1
5	5.31	11.1	102	122	15	13	143	203	98	168	298	389	0.4	0.9	1.00	0.92	7.97	6.95	651	280	3.5	4.6
6	8.18	5.31	88.3	61.6	57	46	125	66.4	13	10	14800	16300	136	146	1.67	1.83	7.74	8.22	873	860	2.1	2.2
7	6.70	5.57	85.2	80.5	92	83	190	173	25	12	1540	1340	8.7	7.9	4.50	4.41	8.12	8.21	898	900	2.1	2.7
8	2.98	4.36	49.0	60.2	35	54	100	118	19	9.2	733	1140	2.6	5.8	3.08	5.00	8.19	7.73	820	911	2.3	2.9
9	9.42	13.1	120	180	49	34	99	131	18	22	1240	1360	2.9	1.8	0.67	0.58	8.01	8.04	748	563	3.0	3.9
10	2.70	2.54	55.7	52.2	155	47	152	89	42	28	1960	674	4.9	2.3	0.92	0.67	8.34	7.42	609	648	3.7	4.0
11	2.46	1.92	23.0	29.7	29	—	51.0	35	17	31	521	395	2.0	2.4	0.75	0.67	7.93	7.89	904	896	2.0	2.8
12	2.43	2.02	68.4	64.7	2.4	2.9	105	69	34	41	174	174	0.6	0.4	0.50	1.08	7.68	7.97	884	871	2.0	2.3
Max:min	13	63	8.1	15	64	39	11	25	25	33	85	160	339	366	9.0	29	22[b]	31[b]	4.9	4.6	2.7	2.6

[a]Sampling date: A90, August 1990; O90, October 1990; J91, July 1991; A91, August 1991.
[b]Ratio calculated for $[H^+]$, not pH.

With this set of predictor variables, all possible subset regression models were computed. The best models were selected on the basis of Mallow's C_p statistic and the adjusted coefficient of determination. The presence of outliers was investigated using the Bonferroni correction for the *t*-statistics for outlier tests (software used: Statistix 3.1). Only models for which all the variables had regression coefficients (slopes) significantly different from zero ($p(t) < 0.05$ were retained.

Results and Discussion

Metal Contamination of Sediments

For all metals, maximum levels of potentially available sediment-bound metals $\{M_s\}$ were found in Lake St. Louis (Table 2). Sediment metal levels showed considerable spatial variability within each lake, and as a result, mean metal levels in the sediments of both lakes were not stastitically different for any metal (*t*-tests for unequal samples: $p > 0.05$). The 22 stations chosen in both lakes cover an important range of metal contamination for all metals. For similar seasons, i.e., August 1990 (Lake St. Pierre) and August 1991 (Lake St. Louis), CVs for metal levels in sediments ranged from 54% for Ni to 300% for Cd. The ratios between maximum and minimum metal levels in the sediments (max:min) were calculated for each metal (Table 2). These ratios clearly indicate that both lakes exhibit an extensive spatial contamination gradient for all metals, the smallest gradient covering levels varying by a factor of 4 ($\{Fe_s\}$, Lake St. Pierre). In Lake St. Pierre, the greatest concentration ranges were observed for $\{Pb_s\}$ (max:min = 71–76) and $\{Cd_s\}$ (max:min = 31–35). In Lake St. Louis, concentration ranges were greatest for $\{Zn_s\}$ (max:min = 85–160) and $\{Cd_s\}$ (max:min = 339–366), mainly due to the presence of very high levels at station 6, near Melocheville. In both lakes, dissolved Ca and DOC were less variable than sediment-related measurements.

Using Eq. 2 and 3, free-metal ion concentrations were calculated for Cd, Cu, Ni, Pb, and Zn (data not shown). CVs ranged from 118% for Pb to 153% for Ni.

Metal Concentrations in Amphipods

Amphipod concentrations of Pb (CV = 273%), Cd (CV = 75%), Fe (CV = 59%), Ni (CV = 55%), and Mn (CV = 53%) were variable from one site to another. This range of variation is similar to that described previously for sediments, suggesting that amphipods do not regulate these metals. In contrast, Zn (CV = 23%), and Cu (CV = 18%) concentrations in amphipods were less variable than in the sediments. Similar conclusions can be drawn from the study of the ratios between maximum and minimum amphipod metal concentrations (max:min), these ratios varying from 1.6 to 2.9 for Zn and Cu and from 3.0 and 32.4 for the other metals (Table 3).

Whereas maximum sediment metal concentrations $\{M_s\}$ were found in Lake St. Louis (Table 2), the opposite trend was found for amphipod metal concentrations, where maximum levels of Fe, Mn, Pb, Ni, and Cu occurred in Lake St. Pierre (Table 3). The discrepancies between both trends emphasize the need to consider the effects of factors other than $\{M_s\}$ on metal bioavailability.

The highest amphipod metal levels observed in this study were similar to those reported for other gammaridean populations from moderately contaminated freshwater ecosystems (e.g., Zauke 1982a; Howell 1985; van Hattum et al. 1991). Zn and Pb maximum levels were approximately 20 times lower than those observed by Rainbow and Moore (1986) in gammarids from Scottish streams located in metal-rich areas. Concentrations of Cd of 2 $\mu g \cdot g^{-1}$ were found at station 1 (Lake St. Louis) — this level of Cd contamination is generally associated with industrial contamination (Stephenson and Mackie 1988). However, metal concentrations in the sediments of station 1 (Table 2) were not very elevated compared with other sites (e.g., station 6, Lake St. Louis). We hypothesize that these high levels of Cd in amphipods are linked to low levels of dissolved Ca in the water at station 1 (Table 2), Ca being thought to compete with Cd for uptake sites on the gills (Stephenson and Mackie 1988). The low levels of dissolved Ca observed at that station are due to the inflow of soft and brown waters from the Ottawa River via Lake des-deux-Montagnes (Fig. 1).

Amphipods were not allowed to depurate their gut before freezing, even though feces are known to contribute significantly to body concentrations of some metals in amphipods (Rainbow and Moore 1986; Amyot 1992) and other aquatic invertebrates (Hare et al. 1989). In parallel experiments (Amyot 1992), Fe, Pb, Ni, and Mn concentrations in feces were shown to alter total metal concentrations significantly in *G. fasciatus*, although this was not the case for Cu, Zn, and Cd. For amphipods to be suitable biomonitors, any laboratory handling after collection must be kept simple to avoid contamination. If depuration is used, it must be complete. However, complete depuration of amphipods can take varying lengths of time, up to 10 d (Rainbow et al. 1989), and is not easily monitored. Depuration might also induce a loss of tissue metal from the organism over such a period (Hare et al. 1989). For these reasons, depuration was not used as a routine procedure in the present study. Moreover, the fact that amphipods were not depurated provides additional information on the possible role of these organisms as pollutant vectors from benthos to nekton, since fishes eat amphipods with their gut contents.

Effect of Size Class and Site on Amphipod Contamination

To assess the influence of amphipod sizes and sampling sites on metal accumulation, we used Friedman's two-way analysis of variance (Table 4). The results indicated a highly significant effect of the site on metal concentrations in amphipods, even when size effects were taken into account. The site influence was weaker with Zn ($p = 0.016$) than for the other metals. Since amphipod metal concentrations were dependent on sample site, *G. fasciatus* apparently did not regulate any of the studied metals in a way that would preclude its use as a biomonitor.

The influence of size classes was also significant for most metals, exceptions being Mn and Ni (Table 4). For all metals except Zn, mean ranks decreased with increasing size classes. Thus, except for Zn, bigger animals were less contaminated than smaller ones. This general trend has been observed for Cu, Zn, Fe, and Pb in other amphipods (Rainbow and Moore 1986). It is possible that smaller animals, displaying a higher surface area to volume ratio, may be more efficient in taking up metals than larger animals. Also, according to Rainbow and Moore (1986), relatively more metal could be sorbed to the cuticle of small organisms. In fact, negative relationships between insect body size and trace metal concentrations have been taken to indicate that a large proportion of the

TABLE 3. Range of amphipod metal concentrations for all sampling sites and each sampling period for both lakes.[a]

Lake St. Pierre sites	Mn ($\mu g \cdot g^{-1}$)		Fe ($\mu g \cdot g^{-1}$)		Pb ($\mu g \cdot g^{-1}$)		Ni ($\mu g \cdot g^{-1}$)		Cu ($\mu g \cdot g^{-1}$)		Zn ($\mu g \cdot g^{-1}$)		Cd ($\mu g \cdot g^{-1}$)	
	A90	O90	A90	O90	A90	O90	A90	O90	A90	O90	A90	O90	A90	O90
1	47–87	69–72	761–1090	265–458	2.0–6.7	0.7–1.1	2.9–3.5	1.1–1.2	60–74	73–79	56–67	67–72	0.29–0.32	0.27–0.32
2	64–66	48–61	1110–1320	381–668	5.3–16.0	0.8–1.9	2.2–5.9	1.1–1.6	77–84	96–123	61–70	73–88	0.22–0.34	0.26–0.31
3	121–130	87–107	772–1280	724–945	8.1–13.7	3.4–3.9	3.1–4.3	2.6–2.7	71–129	71–91	62–79	71–79	0.23–0.28	0.14–0.23
4	173–217	80–104	782–950	324–430	28.6–64.8	1.0–2.0	2.4–2.7	0.9–1.1	68–74	62–72	68–86	72–91	0.35–0.46	0.50–0.55
5	275	80–100	2610	356–550	8.5	1.2–1.5	5.1	1.2–1.5	127	88–97	111	70–73	0.42–0.76	0.38–0.48
6	154	146–175	1270	481–544	3.2	0.7–1.1	2.3	0.9–1.0	105	81–106	83	80–84	0.32–0.39	0.16–0.23
7	—	218–292	—	891–1170	—	2.1–3.5	—	2.0–2.4	—	67–76	—	89–100	0.35–0.46	0.65–0.73
8	—	52–56	—	487–592	—	0.9–1.1	—	—	—	56–71	—	83–104	0.38–0.46	—
9	—	51–67	—	329–479	—	0.5–0.9	—	0.9–1.5	—	75–81	—	76–78	0.38–0.59	0.42–0.58
10	160	—	2430	421–495	2.5	—	1.4	—	68	61–65	60	110–141	0.32–0.44	—
Max:min	5.9	6.0	3.4	4.4	32.4	7.8	4.2	3.0	2.2	2.2	2.0	2.1	3.5	5.2
Lake St. Louis sites	**J91**	**A91**	**J91**	**A91**	**J91**	**A91**	**J91**	**A91**	**J91**	**A91**	**J91**	**A91**	**J91**	**A91**
1	228–249	232–272	637–729	444–667	1.7–2.1	0.3–0.5	1.2–1.8	1.2–1.5	64–71	76–79	76–86	66–85	1.31–2.00	1.65–2.05
2	52–71	73–81	281–330	663–672	0.6–0.8	0.5–0.6	1.2–1.4	1.6–1.8	68–73	85–101	70–83	66–79	0.33–0.51	0.29–0.34
3	107–113	77–94	649–698	396–403	0.6	0.4–1.6	1.8	1.6–1.8	84	89–96	66	61–76	0.36	0.23–0.31
4	141–142	177–187	900–939	629–841	1.9–2.6	0.2–0.7	0.8–1.1	0.9–1.4	78	66–73	66	63–77	0.44–0.51	0.38–0.64
5	—	125–134	—	1170–1450	—	0.8–1.0	—	1.6–2.1	—	65–80	—	79–85	—	0.35–0.70
6	182–183	183–268	652–816	959–1120	0.8–1.4	0.9–1.7	1.1–1.8	1.5–2.2	86	81–89	142–165	124–130	0.82–1.25	0.46–0.83
7	91–147	65–73	636–939	214–353	1.2–1.5	0.4–0.5	0.3–1.4	1.1	69–74	67–76	60–66	72	0.35–0.41	0.13–0.21
8	101	106–111	681	213–267	1.2	0.3–0.8	0.7	0.7–0.9	82	70–73	67	73–76	0.67	0.13–0.16
9	—	107–131	—	393–537	—	0.4–0.7	—	0.9	—	60–65	—	71–76	—	0.27–0.46
10	79–119	176–188	305–438	664–779	0.6–0.9	0.5–1.1	1.0–1.4	1.3–1.5	64–89	65–84	56–85	75–79	0.40–0.67	0.26–0.68
11	—	124–139	—	366–397	—	0.8	—	1.6–2.0	—	80–86	—	73–77	—	0.11–0.19
12	34–51	26–36	326–439	250–303	0.1–0.3	0.1	1.1–1.3	1.1	58–90	72–79	65–71	69–75	0.51–1.01	0.15–0.24
Max:min	7.3	10.5	3.3	6.8	26	17	6.0	3.1	1.6	1.7	2.9	2.1	5.7	18.6

[a]Sampling data: A90, August 1990; O90, October 1990; J91; July 1991; A91, August 1991.

TABLE 4. Friedman's test for randomized blocks assessing effects of size classes and sampling sites on metal concentrations in amphipods. Only stations with values of metal concentrations for each size class were considered.

		Size effect				Site effect	
Metal	N	Size class (mm)	Mean rank	χ^2	p[a]	χ^2	p[a]
Mn	23	3–7	2.39	5.30	ns	63.38	***
		7–9	1.83				
		9–12	1.78				
Fe	23	3–7	2.70	17.04	***	61.29	***
		7–9	1.74				
		9–12	1.57				
Pb	22	3–7	2.50	12.09	**	59.19	***
		7–9	2.05				
		3–7	1.46				
Ni	22	3–7	2.34	4.35	ns	55.22	***
		7–9	1.93				
		3–7	1.73				
Cu	23	3–7	2.61	17.04	***	49.51	***
		7–9	2.00				
		9–12	1.39				
Zn	21	3–7	1.57	9.81	**	35.91	*
		7–9	1.91				
		9–12	2.52				
Cd	22	3–7	2.50	14.82	***	57.18	***
		7–9	2.14				
		9–12	1.36				

[a]p value: ns, not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

TABLE 5. Variations of amphipod metal concentrations (log $[M]_{org}$) between seasons (Lake St. Pierre: August 1990) (x) versus October 1990 (y)) and within a season (Lake St. Louis: July 1991 (x) versus August 1991 (y)) (t-test for paired comparisons with Bonferroni correction).

Metal	Range of log $[M]_{org}$ (min, max)		N	Mean differences ($x - y$)	t value	p[a]
	August 1990 (x)	October 1990 (y)				
Mn	1.68, 2.44	1.68, 2.47	13	0.12	2.37	ns
Fe	2.88, 3.42	2.42, 3.07	14	0.35	6.00	***
Pb	0.30, 1.81	−0.32, 0.59	13	0.80	5.83	***
Ni	0.15, 0.77	−0.08, 0.43	13	0.37	8.26	***
Cu	1.78, 2.11	1.76, 2.09	14	0.01	0.44	ns
Zn	1.75, 2.04	1.83, 2.15	13	−0.01	−0.61	ns
Cd	−0.67, −0.12	−0.86, −0.14	23	0.01	0.23	ns
	July 1991 (x)	August 1991 (y)				
Mn	1.53, 2.40	1.42, 2.43	21	−0.02	−0.58	ns
Fe	2.45, 2.97	2.33, 3.16	21	0.04	0.74	ns
Pb	−0.95, 0.42	−1.08, 0.27	19	0.27	3.02	ns
Ni	−0.46, 0.26	−0.15, 0.33	19	−0.05	−1.59	ns
Cu	1.76, 1.95	1.77, 2.01	18	−0.02	−1.51	ns
Zn	1.73, 2.22	1.78, 2.11	18	−0.01	−0.35	ns
Cd	−0.49, 0.30	−0.97, 0.31	20	0.21	3.67	*

[a]p value: ns, not significant; *$p < 0.0036$; **$p < 0.0007$; ***$p < 0.00007$.

metals is adsorbed externally (Smock 1983). In our study, this latter hypothesis would presumably only apply to Pb, since only this metal is found in high concentrations in the cuticle of *G. fasciatus* (Amyot 1992). Similar conclusions were reached by Hare et al. (1991a) who found that much of the Pb body burden in a burrowing mayfly was located externally

in association with iron oxides whereas Zn and Cd were located internally. Size-dependent variations could also be attributed to changes in feeding habits as a function of size, since individuals were analysed along with their gut contents. An alternative hypothesis could be that the relative volume of the intestinal tract is larger in smaller amphipods than in bigger ones. Since metal concentrations are usually higher in the feces than in the tissue (Amyot 1992), smaller amphipods would thus appear to be more contaminated.

Temporal Variations in Amphipod Metal Levels

To evaluate the potential effect of seasonal change on metal levels in amphipods, a *t*-test for paired comparisons (with Bonferroni correction) was used, in which the pairs were formed of data from August and October 1990 in Lake St. Pierre (Table 5). Highly significant ($p < 0.00007$) differences were found between amphipod concentrations of Fe, Pb, and Ni for different seasons. These differences were not significant ($p > 0.0036$) for Mn, Cu, Zn, and Cd. These variations are likely due to seasonal differences in feces concentrations of Fe, Pb, and Ni (cf. the earlier discussion of the effect of feces on metal burdens — these same metals were those for which the feces contribution was the most important). Such seasonal variations may be attributable to a shift in feeding habits by amphipods as a result of macrophyte bed degradation during the fall. These differences may also be partly due to changes in environmental conditions such as pH, dissolved Ca, DOC, and turbidity (Table 2), resulting from the fall mixing of the water column.

Since amphipod metal concentrations display important seasonal variations in some cases, it seems prudent to limit their use as biomonitors to one season (e.g., summer). To evaluate the importance of summer variations in amphipod metal concentrations, results from July 1991 were compared with those of August 1991 (Lake St. Louis). Results indicated significant (*t*-test: $p < 0.0036$) differences for Cd, amphipods sampled in July being more contaminated than those collected in August (Table 5). It is unlikely that these variations are linked to a shift in environmental conditions, the latter being relatively constant (Table 2). In fact, between July and August, the small variations in water and sediment chemistry (such as an increase in DOC and Cd in sediments and a decrease in dissolved Ca and pH) would tend to enhance Cd bioavailability (Table 2). We suggest that the variations in amphipod Cd concentrations are perhaps linked to changes in the physiological state of the studied populations. For example, levels of Cd in amphipods are influenced by moulting, since Cd can replace Ca in some metabolic pathways (Wright 1980), such as those involved in the amphipod moulting cycle (Graf and Meyran 1983). It is therefore likely that Cd is lost with the cast of exuvia. Furthermore, according to Dumouchel (1969), *G. fasciatus* displays two annual peaks of reproduction in Lake St. Louis, one in June and the other in August. Each event is followed by a period of intensive moulting, which lasts approximately 1 mo (Clemens 1950). Thus, it is plausible that we have sampled more freshly moulted organisms (with lower Cd concentrations) in August than in July. The in situ influence of the moulting cycle on Cd concentrations in gammaridean amphipods has already been observed by Zauke (1982b).

These observed temporal variations in amphipod contamination clearly indicate the need to consider the effect of season in any biomonitoring program using *G. fasciatus*.

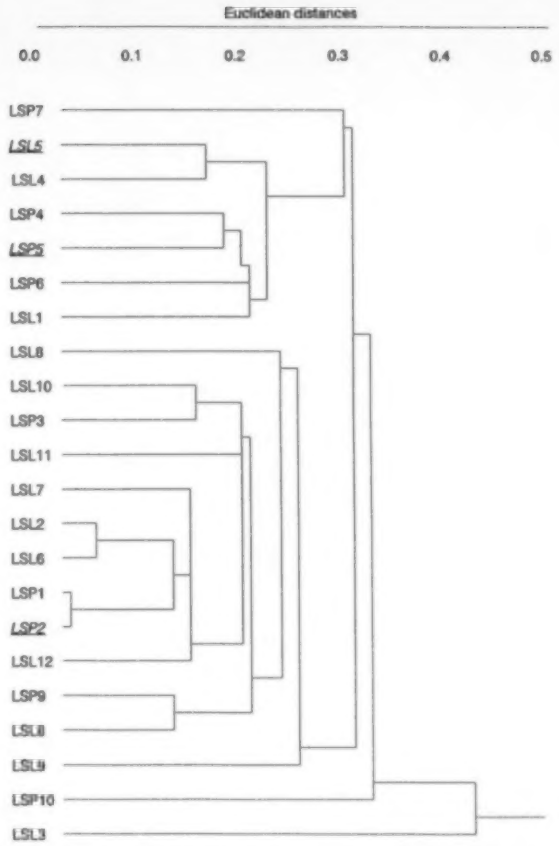

FIG. 2. Dendrograms from a hierarchical cluster analysis linking sampling sites as a function of the similarity of their environmental conditions ($[H^+]$, $[Ca^{2+}]$, $\{Fe\}_s + \{Mn\}_s$, $\{C_{org}\}$, and DOC). The distance metric is expressed in normalized euclidean distance and the single linkage method has been applied. LSP, Lake St. Pierre; LSL, Lake St. Louis. Stations discarded from the regression analysis are underlined.

Modelling of Metal Concentrations in *G. fasciatus*

In order to use an organism as a biomonitor, it is important to understand the origin of its observed metal concentration (Rainbow et al. 1990), i.e., we should be able to predict its metal bioaccumulation as a function of environmental variables. However, this procedure is not straightforward, since organisms can accumulate metals either from the water or from food particles. Also, uptake from both sources will be influenced by physicochemical factors (Tessier et al. 1993). In act, direct relationships between trace metal levels in water or sediments and concentrations in aquatic organisms are seldom encountered in field surveys (Luoma 1989). In a first attempt to assess the significance of trace metal concentrations in *G. fasciatus*, multiple linear regression models were constructed. Only metals of direct ecotoxicological importance (Cu, Cd, Ni, Pb, and Zn) were included.

A cluster analysis was performed using a subset of abiotic variables ($[H^+]$, $[Ca^{2+}]$, $\{Fe\}_s + \{Mn\}_s$, $\{C_{org}\}$, and DOC) associated with each station (Fig. 2). This analysis was conducted to identify stations that were clustered together,

TABLE 6. Multiple linear regression models predicting (log-transformed) trace metal levels ($\mu g \cdot g^{-1}$) in amphipods.[a]

Variables	Coefficients	±SE	t	$p(t)$	Partial R^2	R^2
Cadmium						
Constant	−0.033	0.053	−0.62	0.5381		0.76
$[Ca^{2+}]$	−784	76	−10.35	<0.0001	0.41	
$[H^+]$	-2.41×10^6	4.15×10^5	−5.80	<0.0001	0.20	
$[Cd^{2+}]$	2.05×10^9	2.72×10^8	7.54	<0.0001	0.12	
Size (3–7 mm)	0.06	0.03	2.16	0.0372	0.02	
$N = 37$	$F = 12.21$	$p(F) < 0.0001$				
Copper						
Constant	2.018	0.035	57.14	<0.0001		0.61
$[H^+]$	2.12×10^6	3.29×10^5	6.44	<0.0001	0.42	
Size (7–9 mm)	0.05	0.02	3.00	0.0053	0.10	
Size (3–7 mm)	0.07	0.02	4.12	0.0003	0.08	
DOC	−0.065	0.011	−6.05	<0.0001	0.01	
$\{C_{org}\}$	−16.82	4.87	−3.45	0.0016	0.01	
$N = 37$	$F = 12.21$	$p(F) < 0.0001$				
Lead						
Constant	5.021	0.874	5.74	<0.0001		0.73
DOC	−0.886	0.139	−6.39	<0.0001	0.42	
$\{Pb_s\}$	2.04×10^7	3.37×10^6	6.06	<0.0001	0.15	
$\{C_{org}\}$	−346	90	−3.83	0.0007	0.13	
$[Ca^{2+}]$	−3790	696	−5.44	<0.0001	0.03	
$N = 33$	$F = 22.86$	$p(F) < 0.0001$				
Nickel						
Constant	1.877	0.163	−10.45	<0.0001		0.81
$[Ni^{2+}]$	9.30×10^7	1.11×10^7	8.40	<0.0001	0.43	
$\{Fe_s\} + \{Mn_s\}$	−5754	595	−9.66	<0.0001	0.26	
$[H^+]$	-1.43×10^7	1.48×10^6	−9.63	<0.0001	0.10	
$[Ca^{2+}]$	−1893	181	−10.45	<0.0001	0.03	
$\{Ni_s\}$	2.39×10^6	5.09×10^5	4.69	0.0001	0.01	
$N = 36$	$F = 30.84$	$p(F) < 0.0001$				
Zinc						
Constant	1.62	0.05	30.10	<0.0001		0.68
DOC	0.036	0.009	4.02	0.0004	0.32	
Size (3–7 mm)	0.04	0.008	−4.81	<0.0001	0.18	
$[Zn^{2+}]$	2.78×10^6	7.05×10^5	3.95	0.0005	0.16	
$[Ca^{2+}]$	186	40.3	4.61	0.0001	0.01	
$N = 33$	$F = 17.74$	$p(F) < 0.0001$				

[a]Abbreviations: coefficiients, estimate of partial regression coefficients; ±SE, standard error on estimate; t, t statistic for estimate of partial regression coefficients; $p(t)$, significance level of t statistic; partial R^2, partial coefficient of determination; R^2, coefficient of multiple determination; N, number of observations; F, overall variance ratio from analysis of variance; $p(F)$, significance of F value.

displayed similar environmental conditions (euclidean separation distance less than 0.2), and were less than 5 km apart (Fig. 1). Stations displaying such characteristics were considered likely to be spatially autocorrelated. Three stations were identified: in Lake St. Pierre, stations 2 (similar to station 1) and 5 (similar to stations 4 and 6) and in Lake St. Louis, station 5 (similar to station 4). These three stations were discarded from the subsequent regression analysis.

For the modelling of metal accumulation in amphipods, we have first selected a set of variables derived from the deterministic approach developed by Tessier et al. (1993) for the filter-feeding bivalve *Anodonta grandis*. In this approach, concepts derived from the free-ion activity model of metal–organism interactions, and from surface complexation theory, are used to relate metal concentrations in amphipods to those in the water or in the surficial oxic sediments. Abiotic variables ($[H^+]$, $[Ca^{2+}]$, $[M^{z+}]$, $\{M\}_s$, $\{Fe\}_s + \{Mn\}_s$, $\{C_{org}\}$, and DOC) and amphipod size class have been included in the statistical analysis, since they have been shown to influence metal levels in amphipods. Multiple linear regression was used to evaluate the significance of the contribution of each variable. After selection of the final model, the partial regression coefficients were estimated for the included variables. For each metal, Table 6 summarizes the variables selected, the partial regression coefficients, and the relevant statistics.

For all metals, highly significant coefficients of multiple correlation (R) were found, with values ranging from $R = 0.78$ for Cu to $R = 0.90$ for Ni ($p < 0.001$). The variance explained by the model ranged from 61% for Cu to 81% for Ni (Table 6). For each metal, a different set of variables was selected. Further examination of the partial R^2 for each variable (Table 6) revealed that the variance explained by a given variable was different for each trace metal.

For the same set of data used to compute these models, sediment metal contamination alone could only explain 0–4% ($R^2 = 0.00–0.04$; $p(F) > 0.05$) of the observed variations in Zn, Cu, and Ni amphipod concentrations. Sediment metal alone was somewhat more successful in explaining the variations in amphipod Pb ($R^2 = 0.19$; $p(F) = 0.0064$) and Cd levels ($R^2 = 0.09$; $p(F) < 0.0281$). These observations stress the importance of considering complementary factors that affect the bioavailability of sediment-bound metals, such as $[H^+]$, $[Ca^{2+}]$, $[M^{z+}]$, $\{Fe\}_s + \{Mn\}_s$, $\{C_{org}\}$, and DOC.

Cd concentrations in amphipods were related to concentrations of H^+, dissolved Ca, estimated free Cd, and size classes (Table 6). Station 1 of Lake St. Louis was discarded from the analysis because amphipod Cd concentrations were two times higher than at any other site; the data associated with this station were statistically identified as outliers ($p(t) < 0.05$). Dissolved Ca concentrations alone could account for 41% of the total variation in amphipod Cd. The negative sign of the partial regression coefficient for dissolved Ca suggests a competitive effect of this element. This influence of Ca has been previously observed by Wright (1980) who found an equimolar relationship between Ca and Cd uptake in *Gammarus pulex*. Furthermore, Stephenson and Mackie (1988) observed a strong negative effect in situ of Ca on Cd levels in the amphipod *Hyalella azteca* and suggested that Cd^{2+} competes with Ca^{2+} for uptake sites on the gill surfaces. These latter authors also found that the best subset of predictor variables included Ca, Cd, and DOC aqueous concentrations. Our study generally reaches similar conclusions, except for DOC, which was not retained in our model. Finally, the observed negative effect of increased $[H^+]$ on Cd accumulation is supported by many studies on algae, insects, and zooplankton (Campbell and Stokes 1985; Krantzberg and Stokes 1988; Yan et al. 1990). It is thought that H^+ competes with Cd^{2+} for sites of ion uptake in the biota.

For Cu, size classes, H^+, DOC, and sediment organic carbon were selected as important variables, even though together they only accounted for 61% of the variation. $[H^+]$ accounted for 42% of the total variation (Table 6). The positive effect of $[H^+]$ and negative effect of DOC on Cu bioavailability and toxicity to crustaceans have been previously described by Meador (1991), for ranges of values similar to ours. Our in situ study displays similar relationships. Variables related to Cu concentrations in the environment ($[Cu^{2+}]$ and $\{Cu_s\}$) were not selected. Since amphipod copper concentrations were relatively constant (CV = 18%) whereas environmental concentrations were very variables ($[Cu^{2+}]$: CV = 108%, $\{Cu_s\}$: CV = 88%), we hypothesize that *G. fasciatus* partially regulates Cu, at least over the observed range of environmental concentrations. Thus, *G. fasciatus* does not appear to be a suitable biomonitor of Cu in either fluvial lake. For a given value of $\{M_s\}$, amphipod metal levels were inversely related to $\{C_{org}\}$, suggesting a possible role for metal sorption on sediment organic matter (cf. Tessier et al. 1993).

For Pb and Ni, amphipod concentrations were negatively related to dissolved Ca and positively related to sediment-bound metals (Table 6). It is possible that Ca^{2+} competes with Ni^{2+} and Pb^{2+} at the amphipod gill surface, although only a small part of the overall variation could be accounted for by this variable (3% for both metals). For Pb, DOC explained 42% of the overall variation and was negatively related to amphipod concentrations; such a relation would be expected if DOC were acting to complex Pb. For Ni, 43% of the total variation was explained by $[Ni^{2+}]$ alone. $\{Fe\}_s + \{Mn\}_s$ explained 26% of the variation and was negatively related to Ni concentrations in amphipods, suggesting a reduction of Ni bioavailability by binding to iron and manganese oxide phases present in the sediments. However, laboratory experiments are needed to support these hypotheses.

Zn levels in amphipods were positively related to DOC, estimated free Zn^{2+}, and dissolved Ca and to size class (Table 6). Station 6 from Lake St. Louis was discarded from the analysis because sediment Zn concentrations associated with this site were eight times higher than those found at any other site (Table 2) whereas amphipod Zn levels were approximately two times higher than the average. The data from this station were considered as outliers ($p(t) < 0.05$), since they were inflating the R^2 of our model. DOC explained 32% of the overall variation in Zn. The positive regression coefficient for DOC and Ca is not easily explainable. As for Cu, amphipod Zn concentrations were relatively constant (CV = 23%) and it is likely that amphipods partially regulate this metal. Thus, the use of *G. fasciatus* as a biomonitor of Zn should be considered with caution.

Examination of the plots of predicted versus observed concentrations of Cd, Cu, and Zn in *G. fasciatus* reveals a relatively homogeneous scattering of the points associated with the two lakes (Fig. 3). In contrast, for Ni and Pb, two distinct groups of points were observed, amphipods from Lake St. Pierre being clearly more contaminated than those from Lake St. Louis.

Future Refinement of the Proposed Models

Refinement of the proposed models, or formulation of better ones, should include incorporation of biotic variables, as suggested by van Hattum et al. (1991). For instance, variables related to the contamination of food could be considered. Since *G. fasciatus* has a very diverse diet (Clemens 1950), the metal contamination of numerous compartments should be evaluated (namely periphyton, macrophytes, phytoplankton, zooplankton, particulate matter, and organic debris). One difficulty in such an approach would be to evaluate the relative importance of each component in the amphipod diet. As suggested in the present paper, such variables could explain part of the observed seasonal differences in metal amphipod concentrations.

Improvements could also be achieved in the estimation of abiotic variables, notably the free-metal ion concentrations — e.g., for some metals, sediment organic matter appears to act as an important sorption phase and should be considered when estimating $[M^{z+}]$ (Tessier et al. 1993). Furthermore, since physicochemical variables (e.g., conductivity, temperature, colour, turbidity, and pH) are often subject to variations in fluvial lakes due to movement of water masses, it would be pertinent to integrate measures over time instead of using information obtained on a single sampling date. This point is of particular importance when studying metal bioaccumulation, since this process is not instantaneous in nature.

Note too that we have implicitly assumed that our amphipods are sedentary. Thus, another source of variation in our model may be related to the relative mobility and possible drift of amphipods, both phenomena being known to occur in various environments (Hiroki 1977; Marchant and Hynes 1981; Cellot 1989; Lindström 1991). In fact, to our knowledge,

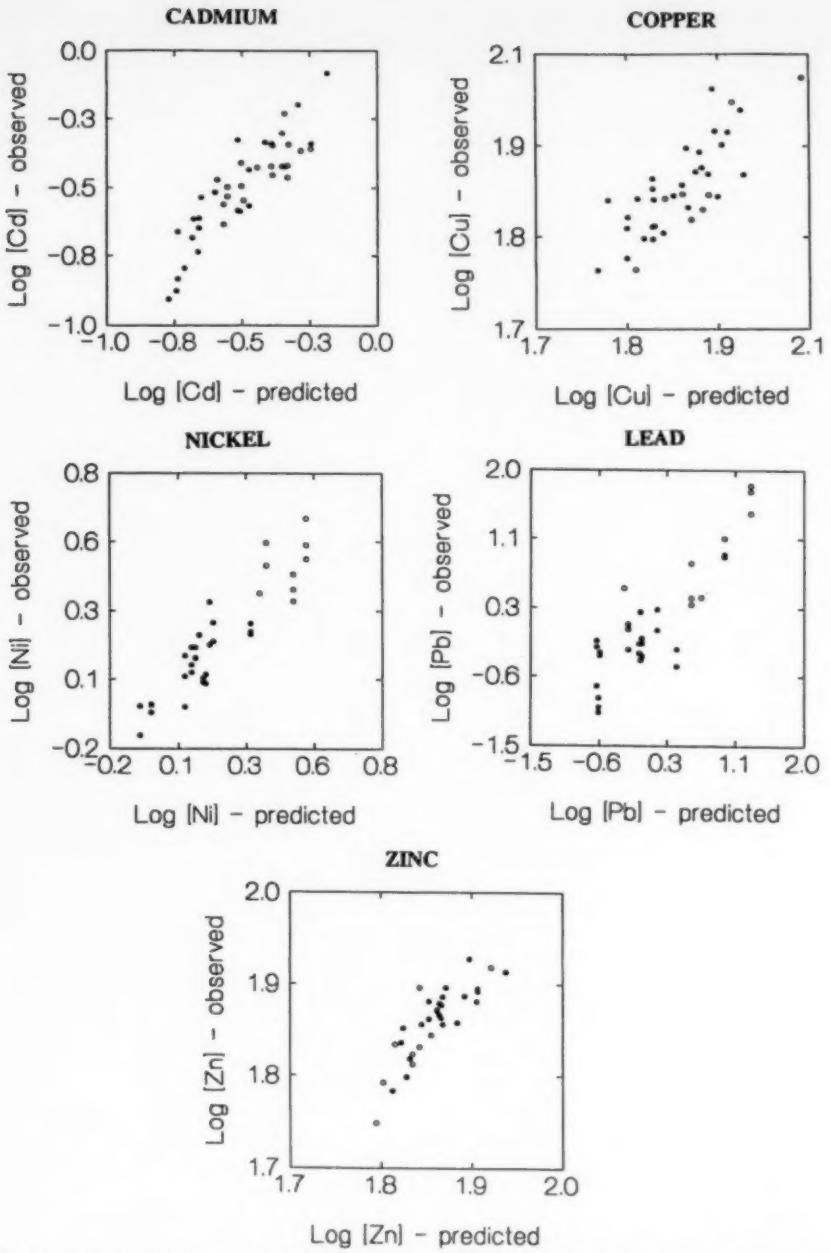

FIG. 3. Relationship between predicted and observed values of log-transformed metal concentrations in amphipods. Predicted values are obtained from multilinear regression models based on a subset of abiotic environmental variables and size classes of amphipods. Open circles represent data from Lake St. Pierre (August 1990) and solid circles represent data from Lake St. Louis (August 1991).

no studies have assessed the impact of these phenomena on amphipod metal concentrations measured at a given location.

Finally, it would be useful to develop an alternative to SCUBA collection if the organisms are to be sampled on a routine basis for biomonitoring. These organisms could be sampled with nets from a boat.

Conclusions

In the present study, we have investigated the potential use of *G. fasciatus* as a biomonitor of trace metal pollution. Our results show that Cd, Ni, and Pb concentrations in this organism can be related to in situ variations of environmental variables of ecotoxicological importance. We suggest

that *G. fasciatus* is a promising biomonitor for these metals. In contrast, Cu and Zn concentrations in the amphipods display only weak within- and between-site variations when compared with variations of sediment concentrations, suggesting the presence of regulating mechanisms. With the exception of Zn, metal body concentrations in smaller amphipods were higher than in larger ones. This trend is consistent with past studies on metal accumulation in amphipods and other small benthic invertebrates.

Seasonal variables have significant effects on Ni, Cd, and Pb concentrations in amphipods. We hypothesize that these seasonal effects could be related to biotic variables such as food contamination and moulting. This temporal variability does not preclude the use of *G. fasciatus* as a biomonitor, but it does impose certain obvious constraints. For example, if spatial variability is to be studied, or if interannual trends are of interest, sampling should be carried out within a narrow time frame.

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Size-related Change in the Visual Resolution of Sunfish (*Lepomis* spp.)

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Walton, W.E., S.S. Easter, Jr., C. Malinoski, and N.G. Hairston, Jr. 1994. Size-related change in the visual resolution of sunfish (*Lepomis* spp.). Can. J. Fish. Aquat. Sci. 51: 2017–2026.

Visual resolution of juvenile sunfish (*Lepomis* spp.) (8–33 mm standard length (SL)), although extremely poor in comparison with the larger individuals (38–160 mm SL) used in previous studies, improves rapidly as they grow. Histologically and behaviorally determined (mean reaction angle) visual angles of fish between 10 and 33 mm SL decrease by approximately 50 and 100 minutes of arc, respectively, and decline non-linearly with increasing fish size. Behaviorally determined visual resolution of juvenile sunfish based on maximum location distance (MLD) is equivalent to that calculated from intercone spacing. The mean reaction angle used in previous studies may have underestimated behavioral visual resolution of larger (>38 mm SL) sunfish by approximately 30%. Visual volume and search volume increase by nearly three orders of magnitude in sunfish between 8 and 50 mm SL. After sunfish exceed 50 mm SL (when they can safely return to the pelagic zone), visual resolution increases comparatively slowly as body size increases. Our results suggest that the size-related change in behavioral visual resolution in sunfish is influenced by other factors in addition to the growth-related changes in the resolving power of the retina.

La résolution visuelle chez de jeunes crapets (*Lepomis* spp.) (de 8 à 33 mm de longueur standard (LS), bien qu'extrêmement faible, comparativement aux individus plus grands (38 à 160 mm de LS) utilisés dans les études précédentes, augmente rapidement à mesure qu'ils grandissent. Les angles visuels (angles de réaction moyens) déterminés par voie histologique et comportementale chez des poissons de 10 et 33 mm diminuent respectivement d'environ 50 et 100 minutes d'arc et baissent de façon non linéaire avec la taille des poissons. La résolution visuelle de jeunes crapets déterminée par voie comportementale et fondée sur la distance maximale de localisation (MLD) est équivalente à celle calculée à partir de l'espacement interconique. L'angle moyen de réaction utilisé dans les mesures précédentes de résolution visuelle comportementale de crapets plus grands (>38 mm de LS) a peut-être été sous-estimé de 30 %. Le volume visuel et le volume de recherche sont devenus 1 000 fois plus élevés chez des crapets de 8 et 50 mm de LS. Une fois que les crapets ont dépassé 50 mm de LS (ils peuvent alors retourner sans danger dans la zone pélagique), la résolution visuelle augmente, mais plus lentement que la taille corporelle. Nos résultats laissent supposer que la variation de résolution visuelle comportementale chez le crapet est fonction d'autres facteurs, en plus de ceux liés à la variation du pouvoir de résolution de la rétine, associée à la croissance.

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Size-related changes in visual resolution (Hairston et al. 1982; Breck and Gitter 1983; Li et al. 1985) and search behavior (Brown 1985; Browman and O'Brien 1992a; Wanzenböck 1992) of fish have important implications for measuring prey selectivity, interpreting predator behavior, and testing foraging models. Understanding how ontogenetic changes in visual resolution affect the visual volume, and ultimately prey encounter rate, is relevant to models of feeding ecology and habitat switching (Werner and Hall 1974, 1988; O'Brien et al. 1976, 1989, 1990; Mittelbach 1981, 1984, 1986; Hairston et al. 1982; Breck and Gitter 1983; Werner et al. 1983; Li et al. 1985; Wetterer and Bishop 1985; Walton et al. 1992). Bluegill (*Lepomis macrochirus*) exhibit ontogenetic niche shifts that are the result of balancing predation risk and foraging returns (Mittelbach 1981, 1984, 1986; Werner and Hall 1988). O'Brien et al. (1989) suggested that the saltatory search behavior observed in bluegill (Janssen 1982; Ehlinger and Wilson 1988) and several other species of planktivorous fish (O'Brien and Evans 1991) is an adaptation to the conflicting demands of predator vigilance and foraging. The geometry of the space scanned for prey by saltatory searching fish is species specific and varies with environmental conditions, prey type, and predator age (O'Brien et al. 1989; Browman et al. 1990; Browman and O'Brien 1992a).

As planktivorous fish grow, so do their abilities to see and to distinguish among prey that differ in size and form. Because visual resolution is directly related to fish size (Baerends et al. 1960; Blaxter and Jones 1967; Blaxter and Staines 1970; Johns and Easter 1977; Rahmann et al. 1979;

Hairston et al. 1982; Neave 1984; Blaxter 1986; Browman and O'Brien 1992a, 1992b), larger fish search larger visual volumes than do smaller conspecifics (Breck and Gitter 1983; Wanzenböck and Schiemer 1989). In addition to conferring a larger visual volume, the enhanced visual capabilities of large fish are thought to permit a more accurate assessment of prey size, and therefore, of potential energy content and handling time of prey (Li et al. 1985). The ability of large bluegill to maximize foraging returns is important because growth rates are often food limited (Osenberg et al. 1988). The prey detection ability and the visual system of large *L. macrochirus* (37–165 mm standard length (SL)) have been studied using both behavioral measurements, such as reaction distance (Werner and Hall 1974; Vinyard and O'Brien 1976; Luecke and O'Brien 1981; Hairston et al. 1982; Breck and Gitter 1983; Li et al. 1985) and heart-rate conditioning (Hawryshyn et al. 1988), and anatomical measurements, such as retinoscopical (Sivak 1973) or histological examination of the eye (Hairston et al. 1982; Williamson and Keast 1988). Otten (1981) argued that electrophysiological and ethological studies are needed to evaluate the estimates of visual resolution, and to test the theoretical predictions, derived from studies of the retina.

Even though the retina of a teleost fish grows throughout much of the animal's life (Müller 1952; Johns and Easter 1977; Easter 1992), the change in retinal structure and components of the eye can be dramatic in juvenile fish (Blaxter 1975; Fernald 1988; Sivak 1990). The retina is thought to be a limiting factor in the resolving power of the photopic system during most of a fish's lifetime (Otten 1981; Meer and Anker 1984). However, the highly correlated, size-related changes in behavioral and anatomical visual resolution (e.g., the visual angle subtended by the prey at detection and the minimum separable angle calculated from the distance between photoreceptors in the retina, respectively) observed in relatively large individuals (Hairston et al. 1982; Li et al. 1985) do not necessarily occur in juvenile fish. For example, Neave (1984) found that anatomical and behavioral visual resolution improved at very different rates during development in both larval plaice (*Pleuronectes platessa*) and turbot (*Scophthalmus maximus*). The relationship between retinal growth and the visual capabilities of fry and fingerling sunfish has not been studied.

In this paper, we examine the visual resolution of young-of-the-year sunfish (*Lepomis* spp.) (8–33 mm SL) and compare behavioral and histological measures of visual resolution for these fish. We associate the size-related changes in visual resolution to the life history of bluegill in north-temperate lakes. We also examine growth-related changes in lens size and the distance between cone receptors in the retina for 10- to 120-mm SL bluegill. We combine our data for young-of-the-year sunfish with those from previous studies of larger bluegill (Hairston et al. 1982; Breck and Gitter 1983; Li et al. 1985) to examine size-related changes in visual resolution, visual field volume, and search volume across a wide range of fish sizes (8–165 mm SL).

Materials and Methods

Study Organisms

Juvenile sunfish were collected from Dryden Lake, Tompkins County, New York, U.S.A., and maintained in 20-L buckets on a daily ration of mixed zooplankton. Because it is not possible to distinguish morphologically between the fingerlings of closely related *Lepomis* species, electrophoretic analyses utilizing diagnostic loci (Dawley 1987) were used to determine the species composition of the juvenile fish after they were used in feeding trials. The majority (80%) of the fish were bluegill and the remainder were pumpkinseed (*Lepomis gibbosus*). Small bluegill (20–55 mm SL) and pumpkinseed (20–40 mm SL) are ecologically analogous: their diets and habitat utilization are similar (Mittelbach 1984, 1986; Werner and Hall 1988), and the growth rates for young-of-the-year of both species exhibit similar responses to environmental factors (Osenberg et al. 1988). We did not observe two distinct groupings in any of the measurements described below, and therefore assume that the visual capabilities of small bluegill and pumpkinseed do not differ significantly.

Behaviorial Measurements

Reaction distances were measured for 36 fish that were distributed among seven size classes (8, 10, 14, 19, 27, 30, and 33 mm SL). Because individual fish became skittish and did not readily feed, groups of three similarly sized sunfish were used in the predation experiments. Experiments were run in an aquarium (78 cm long × 57 cm wide × 25 cm high) constructed of opaque white plastic and with a grid of 1-cm squares marked off on the bottom. In order to keep the fish and prey within the field of view of the video camera, the fish were retained within a clear plexiglass bottomless enclosure (40.5 cm long × 57 cm wide × 16 cm high) positioned in the center of the aquarium. Water depth was either 2 cm (for fish 8–19 mm SL) or 4 cm (for fish 27–33 mm SL). Light was provided by two 40-W cool-white fluorescent lamps giving ambient light levels at the water surface of 4–4.5 $\mu Ein \cdot s^{-1} \cdot m^{-2}$.

Daphnia pulex were sieved and then sorted by eye and measured (mean length (excluding tail spine) ± SD: 2.0 ± 0.1 or 2.4 ± 0.2 mm for the small and large bluegill, respectively). They were introduced individually into the aquarium by a pipet. Each prey was placed at the end of the enclosure opposite that occupied by the fish and was initially outside the visual field of the fish. Sham introductions were made throughout the experiments by pipetting water without prey. Sham introductions never evoked a response by the fish.

A video camera was positioned 260 cm above the water surface to record the positions of the fish's head and the prey. Reaction distance, d, was defined as the distance between the fish and the prey at the time the fish oriented toward the prey and initiated pursuit (Vinyard and O'Brien 1976). Errors in the measured reaction distance due to parallax were less than 3% (Hairston et al. 1982; Walton et al. 1992). Visual angle (α) was calculated as

$$(1)\quad \alpha = 2 \cdot \arctan(h \cdot (2d)^{-1})$$

where h is the mean diameter of the prey (Hairston et al. 1982; Li et al. 1985) and d is the mean reaction distance. The mean diameter of the prey is approximately 64% of body length, excluding the tail spine.

In order to compare our data for small sunfish with those for larger conspecifics, we duplicated the behavioral methods of Hairston et al. (1982) and Li et al. (1985). Recent studies suggest, however, that the maximum observed pursuit distance (MxPD: Browman et al. 1990) or the maximum location

distance (MLD: O'Brien et al. 1990; O'Brien and Evans 1991) is preferable to using the mean reaction distance to calculate behavioral visual resolution for fish utilizing a saltatory search strategy. The MLD is the outer boundary of the distribution of pursuit length (i.e., reaction distance) within which 90% of the pursuits fall (O'Brien and Evans 1991). We also calculated the visual angle of the MLD and the MxPD for each size category.

Histological Measurements

Retinal anatomy was examined using light microscopy following the methods of Hairston et al. (1982). Retinae from 11 fish (10–18 mm SL) were fixed in Bouin's solution, dehydrated in ethanol, and embedded in paraffin. Sections (10 μm thick) were mounted and stained with hematoxylin and eosin. The minor diameters of the cone ellipsoids in a radial section through the center of the eye were measured at 10 randomly selected sites under a 100× oil immersion lens. The number of cones per square millimetre of retinal surface (retinal planimetric density) was corrected for histological artifacts. The Abercrombie correction (Abercrombie 1946; Konigsmark 1970) was used to obtain the number of ellipsoid centers per 10-μm-thick sample and results were corrected for 30% linear shrinkage (Johns and Easter 1977; Hairston et al. 1982). The average distance between cones was calculated as the reciprocal of the square root of the retinal planimetric density (Easter et al. 1977; Fisher and Easter 1979). Visual angles were calculated from histological data following the methods of Easter et al. (1977).

We confirmed that the size of the retinal field in the very small sunfish is equivalent to that in the larger sunfish used by Hairston et al. (1982) by examining the angular subtense of the retina with respect to the center of the lens in six small sunfish (10–18 mm SL). The eyes were not excised from the head. Following decapitation, the head of each fish was sectioned parallel to the line connecting the center of the lens and retina with a cryostat microtome. Photographs were taken as the center of the eye was approached. The size and position of the lens relative to the retina were determined from photographic negatives in which the lens and the globe were largest following the methods of Easter et al. (1977).

In order to compare histological and behavioral measures of visual resolution, we assumed that the Helmholtz criterion (Helmholtz 1924) described the relationship between retinal structure and visual resolution, and therefore, the intercone spacing derived from retinal anatomy was multiplied by 2 (Tamura and Wisby 1963; Breck and Gitter 1983; Li et al. 1985). The intercone spacing per degree of visual angle was doubled by Li et al. (1985) in their study of larger *L. macrochirus*. By also doing so, we can combine our data for juvenile sunfish with data from Li et al. (1985) and compare visual resolution across a large size range of sunfish (8–165 mm SL). The entire range of fish was examined quantitatively by transforming fish size to the inverse of standard length and fitting visual angle to this variable using least squares linear regression. For the behavioral data, the mean visual angle for fish of a particular size from Li et al. (1985) and this study was used in the regression.

Visual Field and Search Volume

The visual field volume of each sunfish size class was calculated by assuming a spherical visual field with a radius equal to the class-specific MLD multiplied by 0.69. The MLD was corrected because the visual field of sunfish is not perfectly spherical (Luecke and O'Brien 1981). The volume of a three-dimensional reconstruction for the 5% prey location contours illustrated in Luecke and O'Brien (1981) was determined by summation of the average areas for adjacent horizontal slices of the visual field. The areas of horizontal cross sections were determined at 2-cm intervals by digitizing (digitizing pad: Summagraphics, model MM1201, Fairfield, CT 06430, USA; software: 3DED, Laboratory for High Voltage Electron Microscopy, University of Colorado, Boulder, CO 80309, USA). The maximum distance from the fish's eye to the 5% prey location contour in the horizontal plane surrounding the fish is assumed to be equivalent to the MLD. The correction factor is the ratio r_s/MLD where r_s is the radius of a sphere with a volume equal to that of the 5% prey location field. We assumed that the correction factor is independent of fish size. The MLD was not available for the largest fish reported in Li et al. (1985). Because the corrected MLD was found to be nearly equivalent to the mean reaction distance, the latter measure was used as the radius of the visual field volume for sunfish >60 mm SL.

The search volume is a pie-shaped wedge of the visual field that is located rostral to the fish's head and within which approximately 90% of the attacks on prey occur (O'Brien et al. 1989). The search volume of each bluegill size class was estimated as

$$(2)\quad SV = MLD^2 \cdot LH \cdot TH$$

where SV is the search volume, LH is the location height, and TH is equal to one half of the location angle and is measured in radians (O'Brien et al. 1989). The location height is equal to one half of the MLD of the white crappie (*Pomoxis annularis*: O'Brien et al. 1989). We assumed that this relationship holds for the bluegill. The mean location angle for bluegill <35 mm SL was estimated from behavioral observations to be 82°. The location angle of large bluegill is 75° (O'Brien and Evans 1991) and we used this value for sunfish >35 mm SL.

TABLE 1. Reaction distances (SD in parentheses) and maximum location distances for seven size classes of sunfish.

Size (mm SL)	*N*	Mean reaction distance (cm)	Maximum location distance (cm)
8	28	3.2 (0.8)	4.2
10	18	4.0 (1.4)	5.7
14	22	5.7 (1.7)	7.2
19	41	7.4 (2.8)	9.2
27	79	19.0 (6.1)	27.7
30	39	19.7 (6.8)	30.1
33	38	25.1 (5.7)	31.2

Results

Behavioral Measurements: Reaction Distance and MLD

The mean reaction distance increased directly with fish size (Table 1). The mean reaction distance of 33-mm SL fish was nearly eight times that of the fish in the smallest size category. The maximum location distance also increased approximately eightfold across the range of fish examined (Table 1). Mean reaction distances ranged from 66 to 80% of the MLD recorded for fish in each size category.

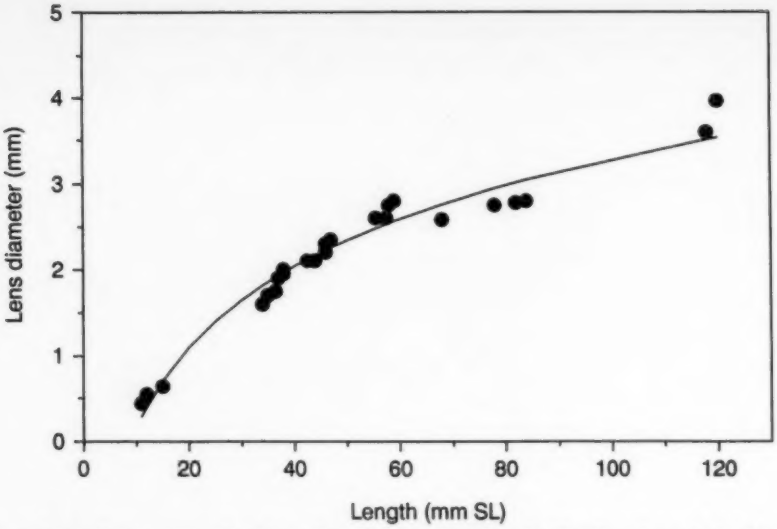

FIG. 1. Lens diameter as a function of sunfish standard length. The line through the data is the best-fitting regression: lens diameter = 1.358(SE = 0.059)·[ln(SL)] − 2.971(SE = 0.228), R^2 = 0.96, RMS = 0.030, N = 24, where R^2 is the coefficient of determination, RMS is the residual mean square for the regression, N is the sample size, and SE is the standard error of the regression coefficient.

Histological Measurements

Lens size and position

Lens diameter increased as a function of body size, but the slope of the relationship declined in large fish (Fig. 1). In 10- to 33-mm sunfish, the increase in lens diameter was approximately linear; lens diameter increased from about 0.5 mm in 10-mm fish to approximately 1.5 mm in 33-mm fish (Fig. 1). Thereafter, lens growth slowed as fish length increased.

The position of the lens center relative to the retina indicated that retinal field size was independent of fish size. In 10- to 18-mm sunfish, the lens center was situated in the plane of the pupil (or slightly medial) and the relationship of the lens center to the plane of the pupil and the lens center to the retina suggested that the retina subtended ≥180° of visual angle. Similar relationships were observed in the sunfish used by N.G. Hairston et al. (unpublished data). Therefore, the retinal angle and retinal magnification calculations used previously for larger sunfish were appropriate for sunfish ≤33 mm SL.

Intercone distance and cone diameter

The average distance between cone receptors in the retina increased linearly with increasing fish size (Fig. 2). The linear distance between the cones in 120-mm fish was 2.2 times that in 10-mm sunfish. Even though the distance between cone receptors increases directly with fish size, the intercone angles decrease with increasing fish size as the retina enlarges and recedes from the lens center during growth (Fig. 3).

The average cone diameter also increased linearly as a function of fish standard length (Fig. 2). The minor diameter of cone ellipsoids increased from about 2.4 to 3.3 μm as sunfish grew from 10 to 60 mm SL.

Comparison of Visual Resolution Determined by Behavioral and Histological Methods

The general shape of the function, visual angle versus fish standard length, was similar for both measures of visual resolution. The visual angle subtended by the prey, at detection, was inversely related to fish size. In both anatomical and behavioral studies, visual angles decreased markedly with increasing fish size for sunfish <20 mm SL (Fig. 3); our anatomically based visual angles were about 50–60% of those calculated using the mean reaction distance from the behavioral studies.

Nearly all of the prey detections by sunfish <20 mm SL required an image size that was greater than twice the intercone interval. The proportion of prey detections with an image size greater than twice the intercone interval declined from >97% in sunfish <20 mm SL to 92 and 66% in 20- and 33-mm SL sunfish, respectively.

The best fits of body size versus anatomical and behavioral measures (based on the mean reaction angle) of visual resolution were quite different (Fig. 4). The regression lines for both measures of visual resolution diverged noticeably for sunfish <20 mm SL. Both the slope and intercept estimates for the two regressions differed significantly (slopes: t_{47} = 13.821, $P < 0.001$; intercepts: $t_{47} = 3.081$, $P < 0.025$).

The variation around the mean behavioral visual angle declined as fish size increased (Fig. 3). The variation in visual angles for 8- to 19-mm SL sunfish was approximately three times (±30–40 minutes of arc) that observed for >25-mm SL fish; however, this trend was opposite that observed for the mean reaction distance (Table 1). Even if the variation around the mean reaction distance of each fish size class were equivalent, the variation around the mean visual angle of small fish would have been larger than the variation around the mean visual angle of large fish because the arctangent function approaches the x-axis asymptotically.

Whereas the visual angles calculated using the mean reaction distance were approximately two times larger than those determined by doubling the intercone distance, visual angles calculated using the MLD or the MxPD were similar to

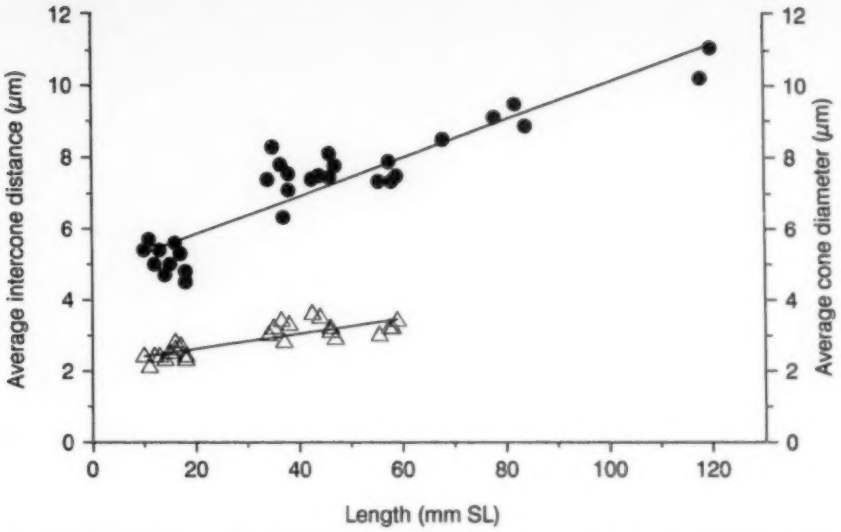

FIG. 2. Average distance between cone receptors (●) and the average diameter of cone receptors (△) in the retinae of sunfish. The best-fitting regressions are shown: average intercone distance = 0.053(SE = 0.004)·SL + 4.793(SE = 0.202), R^2 = 0.86, RMS = 0.405, N = 32; average cone diameter = 2.200(SE = 0.109)·SL + 0.021(SE = 0.003), R^2 = 0.68, RMS = 0.064, N = 25. Abbreviations are the same as in Fig. 1.

those based on twice the intercone distance in the small sunfish (Fig. 3). Behavioral visual resolution based on the MLD or the MxPD also increased nonlinearly with increasing fish size.

Visual Field and Search Volume

Based on a three-dimensional reconstruction (Fig. 5) using the contours of prey location ability from Luecke and O'Brien (1981), the volume of the visual field was estimated to be one third that of a sphere with a radius equal to the maximum reaction distance in the horizontal plane of the fish. It is assumed that the maximum reaction distance for the 5% prey detection contour in the horizontal plane of the fish approximates the MLD. This is a reasonable assumption because the ability of sunfish to detect prey changes rapidly with increasing distance from the fish (cf. 95 and 5% prey detection contours in Fig. 5); the outer boundry within which 90% of the pursuits fall is likely to be close to the 5% prey detection contour. An equivalent spherical volume for the visual field bounded by the 5% prey detection contour has a radius equal to 0.69·MLD. The radius of the visual field approximates the mean reaction distance in the horizontal plane of the fish which, in the small sunfish (Table 1), averaged 74% of the MLD.

Size-related changes in visual resolution, visual field volume, and search volume were greatest for the small sunfish (<20 mm SL). As anatomically based visual angles declined precipitously in small sunfish, visual field volume and search volume increased conversely (Fig. 6). For bluegill viewing a 2-mm *Daphnia*, the volume of the visual field increased from 0.14 L for an 8-mm SL sunfish to nearly 217 L for a 160-mm SL sunfish. The search volume was on average 22% (range 17–26%) of the visual field volume. The search volume of bluegill viewing a 2-mm *Daphnia* increased from 0.027 L for an 8-mm SL fish to nearly 37 L for a 160-mm SL fish.

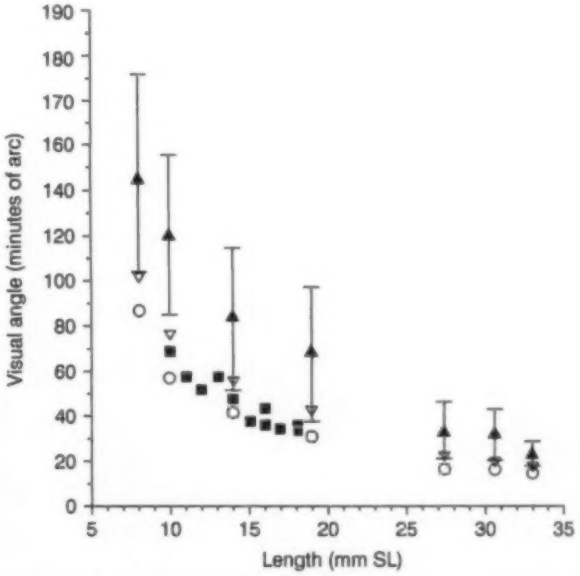

FIG. 3. Visual resolution of juvenile sunfish. ▲, mean visual angle (±SD) calculated from reaction distance; ■, visual angle calculated from histological sections (intercone spacing multiplied by 2); ▽, visual angle calculated from the maximum location distance; ○, visual angle calculated from the maximum observed pursuit distance.

Discussion

Size-related Change of Visual Resolution

The visual resolution of sunfish improves monotonically with increasing body size and changes rapidly in young-of-the-

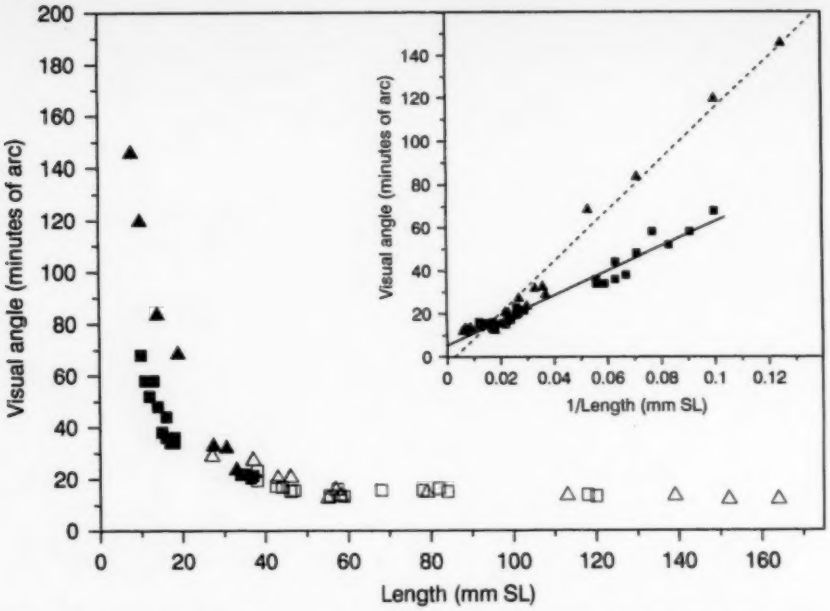

FIG. 4. Change in the visual resolution of sunfish as a function of fish body size. ▲, mean visual angle calculated from reaction distance; ■, visual angle calculated from histological sections (intercone spacing multiplied by 2). The data for fish >35 mm SL (open symbols) were taken from Hairston et al. (1982) and Li et al. (1985). Inset: linear regressions of visual angle on fish standard length^{-1} for behavioral (---, mean reaction angle) and histological (—) measures. Behavioral visual angle = 1188.70(SE = 45.68)·mm^{-1} − 3.58(SE = 2.13), R^2 = 0.98, RMS = 39.273, N = 19. Histological visual angle = 555.57(SE = 22.06)·mm^{-1} + 7.11 (SE = 1.01), R^2 = 0.96, RMS = 10.842, N = 32. Abbreviations are the same as in Fig. 1.

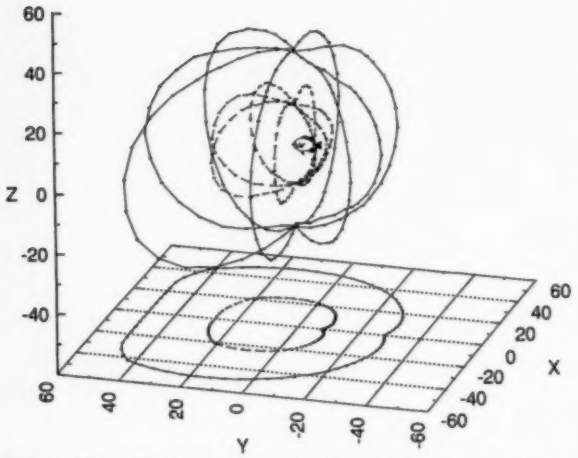

FIG. 5. Visual field of a bluegill (~56 mm SL) searching for a 2-mm *Daphnia* under high light levels (~5900 lx). The three-dimensional reconstruction is based on data for 5- to 9-cm (total length) bluegill in Luecke and O'Brien (1981). The contours circumscribing the regions where prey are detected in 95% of the trials (contour closer to the fish) and in 5% of the trials (contour farther from fish) are illustrated. The projections of both contours onto the x–y plane are illustrated. The fish is positioned at the origin and the scale of the axes is centimetres.

year fish. Visual resolution of the small sunfish (8–33 mm SL) examined both histologically and behaviorally in this study was less acute than that of larger sunfish (≥38 mm SL: Hairston et al. 1982; Breck and Gitter 1983; Li et al. 1985). As sunfish grew from 10 to 38 mm, histologically and behaviorally determined (mean reaction angle) visual angles decreased by approximately 50 and 100 minutes of arc, respectively. The MLD increased more than fivefold during this interval. Behavioral visual resolution based on the MLD was, however, more similar to histological visual resolution based on twice the intercone interval than was behavioral visual resolution calculated using the mean reaction angle.

The marked improvement in visual resolution observed in juvenile bluegill is also observed in other teleosts. At 2 wk after hatching (8 mm SL), bluegill behavioral visual resolution was estimated from the MLD at 1°43′. At about 3 mo after hatching (33 mm SL), behavioral visual resolution was estimated to be 18′. Neave (1984) found that the behavioral visual angles of larval plaice (6–7°) and turbot (5–6°) at the age of first feeding exceeded the minimum separable visual angle based on cone cell spacing (1° and 1°20′, respectively). Behaviorally determined visual angles of both species decreased appreciably during larval development and were smaller at metamorphosis (both species: 11′) than were histologically based visual angles (plaice: 40′, turbot: 20′). In larval Atlantic herring (*Clupea harengus*), a simple retina is retained for months after hatching, and the visual resolution of 10-mm larvae using the unspecialized retina was estimated from histological preparations to be 200′ (Blaxter and Jones 1967). The minimum separable visual angle declined steeply in herring <50 mm and declined more slowly in fish larger than 50 mm (Blaxter and Jones 1967, Blaxter 1975). At

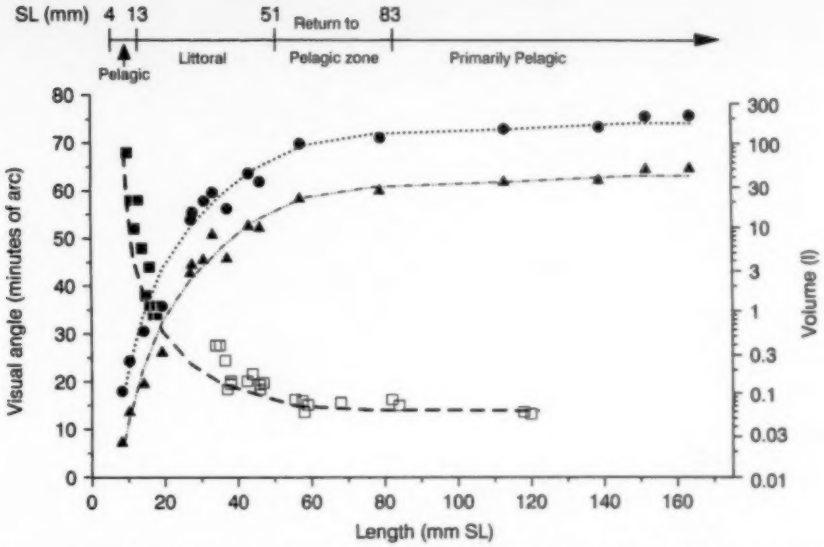
SL (mm) 4 13 51 Return to 83
Pelagic
Littoral
Pelagic zone
Primarily Pelagic
Visual angle (minutes of arc)
Volume (l)
Length (mm SL)
0 20 40 60 80 100 120 140 160
0 10 20 30 40 50 60 70 80
0.01 0.03 0.1 0.3 1 3 10 30 100 300

FIG. 6. Comparison of the size-related change of histologically determined visual resolution (■, this study; □, Hairston et al. 1982; Li et al. 1985) versus visual field volume (●) and search volume (▲) of bluegill searching for a 2-mm *Daphnia*. The visual field volume is assumed to be spherical with a radius equal to the 0.69·MLD (or the mean reaction distance (see text)). The search volume is a rostral, triangular wedge of the visual field as defined by O'Brien et al. (1989). The size-related change in habitat preference for bluegill populations inhabiting small lakes in southwestern Michigan (Werner and Hall 1988) is shown at the top of the figure. The vertical lines and numbers indicate the standard length at which major changes in habitat preference occur.

300 mm, the visual resolution improved to 25′ in the unspecialized region of the retina and was estimated to be 7′ in the area temporalis (Blaxter and Jones 1967). Behavioral visual resolution in larval rainbow trout (*Oncorhynchus mykiss*) improved from 2° shortly after hatching to 18–28′ at 68 d after hatching (Rahmann et al. 1979). Histologically determined visual resolution of yellow perch (*Perca flavescens*) improved from 174′ in newly hatched larvae to 9–12′ in the adult (>200 mm SL: Wahl et al. 1993).

The sharp decline in the histological visual angle as fish grew was accompanied by a comparatively abrupt increase in the visual volume. The eyes of the smallest sunfish have poor visual resolution and are capable of searching only small visual volumes (<1 L: Fig. 6). Bluegill fry remain on the nest for 4–10 d after hatching before leaving the nest at about 4 mm SL to reside in the pelagic zone for a period of 4–7 wk (Werner 1967; Beard 1982; Werner and Hall 1988). At approximately 12–14 mm SL, bluegill fry return to the littoral zone and reside in and near littoral vegetation for several years (Werner and Hall 1988). During the period when bluegill emigrate from, return to, and then reside in the littoral zone, the eye continues to grow and the minimum separable visual angle calculated from intercone spacing declines markedly from approximately 1°10′ in 10-mm SL individuals to 17′ of arc in 50-mm SL sunfish (Fig. 6). Visual volume increases nearly three orders of magnitude, from about 0.1 L at 8 mm SL to 90 L at 50 mm SL.

Hairston et al. (1982) suggested that a selective advantage provided by continuous retinal growth was the improved prey detection abilities of larger fish: the potential of larger retinae to resolve smaller objects. As visual volume increases, larger sunfish are able to discern smaller prey at greater distances and also see more prey than can small fish in the same environment (Hairston et al. 1982). Enhanced visual capabilities are also thought to permit a more accurate assessment of prey size and, therefore, of the potential energy content and handling time of prey (Li et al. 1985). Such improvements in visual resolution are presumably significant for a sunfish to maximize its energetic intake. For example, the inclusion of suboptimal prey in the diet has a smaller total effect on energetic intake as fish size decreases (Mittelbach 1981), suggesting that factors that augment prey selection ability, such as better visual resolution, are particularly important to large sunfish. Further, the growth rates of large bluegill in small Michigan lakes were strongly associated with zooplankton profitabilities and were probably food limited (Osenberg et al. 1988).

Visual resolution changes gradually in large, predominantly planktivorous bluegill (>50 mm SL: Fig. 6); yet small changes in visual resolution result in substantial differences in visual volume as fish grow. After 2–4 yr in the littoral zone, bluegill between 51 and 100 mm SL switch seasonally from the littoral vegetation to the open-water habitat (Mittelbach 1981) or reside almost exclusively in the pelagic zone (Werner and Hall 1988). Werner and Hall (1988) found that bluegill returned to the pelagic zone at sizes between 51 and 83 mm SL, and further that the size at which bluegill returned to the pelagic zone was directly correlated with the abundance of piscivores. Visual resolution changes very little and visual volume increases at a comparatively slow rate after bluegill attain the size (51 mm SL) at which they return to the open-water habitat (Fig. 6). The minimum separable

visual angle calculated from histological preparations decreases from about 17′ in 50-mm SL fish to 13′ in 120-mm SL fish. Even though visual volume increases slowly in bluegill >50 mm SL, the visual volume of a 160-mm SL fish viewing a 2-mm *Daphnia* is 2.3 times larger than for a 50-mm SL fish.

Because the mean (or median) reaction angle was used to estimate the behavioral visual resolution of the larger sunfish used in previous studies (Hairston et al. 1982; Breck and Gitter 1983; Li et al. 1985), behavioral visual resolution was underestimated. If the reaction angle for the MLD is substituted for the mean reaction angle, then the behavioral visual angles of the sunfish used by Hairston et al. (1982) were overestimated by approximately 30% (range 14–44%). Li et al.'s (1985) reanalysis of the data from Hairston et al. (1982) and from several other studies (Werner and Hall 1974; Vinyard and O'Brien 1976; Breck and Gitter 1983) showed that the mean reaction angles from behavioral studies for sunfish between 37 and 120 mm SL were equivalent to anatomically determined visual angles after the latter were doubled in accordance with the Helmholtz criterion. Reaction angles calculated using the MLD are smaller than the mean reaction angles from a particular sunfish size class. The behavioral visual resolution of an adult bluegill is therefore better than the visual resolution calculated from a composite measure of intercone spacing. The distribution of photoreceptors in the retina of adult bluegill is relatively uniform (Williamson and Keast 1988) as compared with marine teleosts (Easter 1992) and other centrarchids (Williamson and Keast 1988; Browman et al. 1990; Cameron and Easter 1993); however, the reaction angle of a 120-mm SL bluegill (10′: mean reaction angle reduced by 30%) is still larger than the minimum separable angle calculated from cone cell spacing in the region of greatest photoreceptor density (5.4′: twice the intercone distance in the dorsotemporal region of the retina reported by Williamson and Keast (1988)). Hairston et al. (1982) cautioned against assigning much significance to the differences between histological and behavioral measures of visual resolution because of uncertainties associated with each measure such as absolute prey size, histological artifacts, and slight intraretinal regional differences that might affect prey detection and the average intercone distance. Methodological differences complicate comparisons among studies. For fish that use a saltatory search pattern, the MxPD and MLD are clearly better measures of the absolute visual capabilities (minimum separable visual angle) than is the mean reaction distance (Browman et al. 1990; O'Brien et al. 1990).

Bluegill concentrate their attacks on prey positioned in a comparatively small space in the visual field, and it is therefore unlikely that the mean reaction distance for such attacks should approximate the radius of a spherical equivalent to the visual field volume. We conclude that the equivalence of the mean reaction distance and the radius of the visual field volume is coincidental. Even though the eyes are positioned laterally and bluegill can detect prey that are positioned caudally in the visual field, attacks on prey located in the caudal hemisphere of the visual field are relatively rare (O'Brien and Evans 1991; this study). The detection ability of bluegill is greatest for prey positioned at 45° rostrally to the longitudinal (rostrocaudal) axis in the horizontal plane of the fish; prey detection ability declines both dorsoventrally and laterally to the fish's head (Luecke and O'Brien 1981). Bluegill and other planktivorous fish concentrate attacks on prey in the forward-directed hemisphere (Dunbrack and Dill 1984; O'Brien et al. 1989, 1990; O'Brien and Evans 1991): a space that, at the low prey densities used here, is only 17–26% of the bluegill's visual field volume. For a cruising predator such as juvenile coho salmon (*Oncorhynchus kisutch*), forward-directed locomotion or the movement of stream water past a stationary fish skews the distribution of encounters to prey located rostrally (Dunbrack and Dill 1984). This encounter bias is less likely to occur for a bluegill that restricts scanning for prey to pauses in locomotion and forages in more quiescent lentic environments. There may be a cost (i.e., energetic or time) to reorientation in a lateral or caudal direction; however, the cost of changing direction or scanning the entire visual field would likely be small relative to the cost of pausing. The cost of braking could be considerable, particularly after fast pursuits (O'Brien et al. 1989). The forward-directed bias of prey attacks may be caused by a forward-directed region of slightly higher visual resolution and a tendency to attack prey that are located within or near a region of binocular vision. Based on the direction of accommodative lens movement, the occurrence of an aphakic space (Sivak 1973), and neurophysiological (Schwassman and Kruger 1965) and behavioral evidence, Wetterer (1989) argued that vision in bluegill is primarily forward directed and, if bluegill require binocular cues to evaluate actual prey size and distance of prey, they would be able to do so only when the prey occurs within the bluegill's binocular field of approximately 40°. The location angles of the small bluegill examined here were, on average, only slightly greater than those of larger conspecifics (O'Brien and Evans 1991); this was unlike the dramatic, size-related change in reaction distance.

Image Size and Quality

Behavioral observations indicate that prey detection by small bluegill requires a large image and suggest that the image might be blurred. Prey detection by very small sunfish (<20 mm SL) required images that, in most instances, were greater than two times the average intercone spacing. The proportion of prey detections with an image size greater than twice the intercone interval declined with increasing fish size for the sunfish examined here and for the intermediate-sized sunfish used by Hairston et al. (1982; <40% in 55- to 58-mm SL bluegill: J.K. Wetterer, Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138, USA, personal communication). Both cognitive factors, such as learning (Meyer 1986) and prey recognition (Hughes 1979), and ontogenetic improvements in the visual system affect the size-related changes of behaviorally determined visual resolution.

Evolutionary solutions that improve visual resolution during development may involve different parts of the visual system in different fish species (see Otten 1981; Muntz 1990). In addition to cone cell size and spacing, resolution increases with eye size and depends on aperture size (pupil diameter), neuronal connectivity, and lens quality (Muntz 1974; Baxter 1975; Fernald 1988; Sivak 1990). Aperture size limits the resolving power of only very small eyes (*Haplochromis elegans*: lens diameter <0.5 mm; Otten 1981). Browman et al. (1990) suggested that the disparity between measures of anatomical and behavioral visual resolution resulted from overestimates of visual resolution by anatomical

measures that fail to account for cone cell convergence onto ganglion cells and higher brain centers (but see Sivak 1990). Collin and Pettigrew (1989) found that visual resolution calculated from ganglion cell spacing was highly correlated with, and was of comparable magnitude to, that based on cone cell density from previous studies of marine teleosts. The degree of neural summation in the retina of the adult bluegill is intermediate among fish in general and low among the centrarchids (Williamson and Keast 1988). Wagner (1978) argued that the significance of spatial visual function (i.e., lateral inhibition) in the visual system is the possibility to compensate for the blurred image of an object on the retina caused by stray light or for a blurred stimulus pattern caused by divergent neural impulses. For young fish, the spatial visual function typically lags behind the structural maturation of the cone cells (Powers and Raymond 1990).

The relative importance of size-related differences in lens quality (Sivak and Bobier 1978; Kruezer and Sivak 1984; Fernald and Wright 1985a; Sivak 1990) and accommodative mechanisms that move the lens and focus the image onto the retina (Sivak 1973; Fernald and Wright 1985b) is unknown for small sunfish. Lens diameter increases rapidly in juvenile fishes and the lens grows more slowly in larger individuals (Zaunreiter et al. 1991; Wahl et al. 1993; this study). In adult fish, resolution of the lens is typically much smaller than the minimum separable visual angles calculated using the distance between photoreceptors in the retina (Northmore and Dvorak 1979; Fernald 1988). Lens quality in several fish species improved with increasing fish size (Muntz 1974) and was often optimal in sexually mature individuals of intermediate age (Sivak 1990). Any of the aforementioned factors could cause blurring of the image on the retinae of small sunfish and merit further investigation. The present study shows that the nonlinearities in behavioral visual resolution are closely associated with changes in the resolving power of the retina, but our results suggest that the size-related change of behavioral visual resolution in sunfish is influenced by other factors in addition to the growth-related changes in cone cell spacing and lens focal length.

Acknowledgments

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Effects of Movement on Stock Assessment in a Restricted-Range Fishery

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A simple spatial model is proposed for yellowfin tuna (*Thunnus albacores*) in the eastern Pacific Ocean for the period prior to the late 1960's, when the fishery was confined to a relatively narrow coastal belt and a few offshore islands and banks. Yellowfin are highly mobile, but they exhibit little or no tendency towards directed movement; their movement can be characterized as highly diffusive. The effect of the fishery extends far beyond the range of the fleet. Within this context the concept of a "stock" is not well defined. Diffusive transport distorts the equilibrium yield – effort curve: an asymmetry is imparted to the Schaefer model. It appears that the catches of the coastal fishery were largely sustained by immigration from the larger offshore region, which constituted a refuge. If the cost of effort is considered as a function of distance from shore, then the expansion of the fishery might be attributed to a change in that function. Costs change with improved technology, and innovation may have been spurred by regulations that were imposed to conserve yellowfin in the eastern Pacific Ocean.

On propose un modèle spatial simple pour l'albacore à nageoires jaunes (*Thunnus albacores*) dans le Pacifique est pour la période antérieure à la fin des années 1960, une époque où la pêche était confinée à une bande côtière relativement étroite et à quelques îles et bancs au large. L'albacore est très mobile, mais il a peu ou pas de tendance à se déplacer de façon orientée; on peut caractériser ce déplacement comme très désorienté. L'effet de la pêche s'étend à une zone beaucoup plus grande que celle parcourue par la flotte. Dans de telles conditions, le concept de « stock » n'est pas bien défini. Le déplacement désorienté a un effet de distorsion sur la courbe rendement d'équilibre – effort : il confère une certaine asymétrie au modèle de Schaefer. Il semble que les captures de la pêche côtière étaient largement compensées par l'immigration en provenance d'une région plus vaste au large, région constituant un refuge. Si l'on considère le coût de l'effort en fonction de la distance du rivage, on peut attribuer l'expansion de cette pêche à une modification de cette fonction. Les coûts varient avec les progrès de la technologie, et des innovations peuvent avoir été suscitées par la réglementation qui a été établie pour conserver l'albacore dans l'est du Pacifique.

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Early fisheries studies such as that of Schaefer (1957) used catch per unit of effort (CPUE) as an index of abundance over time and simply disregarded spatial variation. In this report, these are called aggregate models. Ricker (1944) laid out clearly the assumptions for such neglect to be tenable: either the population must be highly mobile or the distribution of fishing effort must be random. He envisaged individual fish to be moving at random, leading towards a uniform distribution. This would be disrupted by fishing, which would create local reductions in the abundance. The more mobile the fish, the sooner the local anomalies would be eroded by immigration. A nonuniform distribution of fish could still be measured using the overall CPUE if effort were distributed at random. Ricker argued that, for most stocks, the quasi-random distribution of effort, combined with the movement of fish, was sufficient to render any geographic variation in catch rates insignificant.

The distribution of effort for yellowfin tuna (*Thunnus albacores*) in the eastern Pacific Ocean has not been distributed randomly throughout the range of the population. It was confined to the coastal zone and a few offshore islands and banks until the late 1960's, when the area of operation increased by a factor of 6 or 7. Yellowfin are highly mobile, but not relative to the size of their range; their dispersal is insufficient to have maintained homogeneity while the fishery was confined to the coastal zone.

Gulland (1955) saw the variation in catch rates as an example of stratified sampling. He showed that it was unnecessary to assume homogeneity if one took the mean of CPUE of all the strata. If some strata were unsampled, then one could obtain an estimate only for the sampled subpopulation. The potential range of the fishery for tunas in the eastern Pacific Ocean was, of course, unknown prior to its expansion, so the relative abundance could be estimated only for the population available to the contemporary fishery. The increased catches that occurred with the expansion were recognised as evidence for a larger population, and the aggregate model was adjusted accordingly. But a six- or sevenfold increase in area of operation provided only about twice as much sustainable catch (Inter-American Tropical Tuna Commission (IATTC) 1992). I shall argue that this was due in part to migration of fish from offshore to the restricted zone of exploitation and that migration distorted our understanding of the dynamics of the fishery.

The spatial model proposed is similar in concept to that of Fox (1977), who used a Schaefer model for whales, in which there was partial mixing of stocks from two areas. Fox formally allowed for asymmetrical rates of transfer, but in each example the proportion transferred from both populations was equal, so mixing was, in essence, diffusive. He showed that the yield–effort curve could be distorted from the parabola that is obtained with a single population.

Here, a one-dimensional continuum is envisaged, rather than just two stocks, with exploitation depressing abundance in one end-section of that continuum. The mechanism of mixing is assumed to be diffusion. A constant coefficient of diffusion determines that the proportion transferring is everywhere constant. Fish diffuse towards this section, and a gradient of abundance evolves. This migration enhances the catch. The emigration from unfished areas reduces abundance in those areas and stimulates production there. Thus, the area of exploitation is greater than the range of the fishing boats. The extent of this "action at a distance" is determined not only by the mobility of the fish, but also by the population growth rate and by the pressure exerted by the fishery.

A variable area of exploitation creates difficulties for our concept of a stock. If we consider the stock as only those fish that are directly within the range of the fleet, then we ignore a large portion of the population that sustains the catch — and, perhaps, an even greater portion that might sustain increased effort. A larger population must be studied, although parts of its may be almost unaffected by the fishery.

In retrospect, it can be seen that little could be deduced about the population dynamics from catch statistics while the fishery was confined to a small part of the population's range. Growth and immigration are confounded, and the carrying capacity depends upon the effective area of exploitation, which is, in turn, a function of fishing effort, as well as growth rate and mobility.

History of the Fishery

The Schaefer (1954, 1957) model was originally developed for this fishery. The dynamics of the population and the history of the industry made this fishery particularly appropriate to biomass modelling. A long series of reliable catch and effort statistics was available for a period during which the fishing effort had varied considerably. Individual yellowfin grow rapidly and have a high natural mortality rate, so the population responds quickly to any change in fishing mortality.

The Schaefer model assumes logistic growth (Pearl 1925) and adds an additional term to represent fishing. The equilibrium yield is a function of fishing effort and is symmetrical about the point of maximum equilibrium yield. The IATTC, whose role is to provide management advice to its member governments, estimated that the maximum equilibrium yield was surpassed in 1961. A catch quota was first imposed in 1966, by which time the CPUE had declined, but not as expected; it was clear that the equilibrium yield curve was not symmetrical. Pella and Tomlinson (1969) proposed a generalisation of the Schaefer model that allowed for this asymmetry at the cost of introducing another parameter.

Until 1959, baitboats were the mainstay of the fleet. Bait was caught in inshore waters, and the range was restricted to the distance that the bait could be transported alive. During 1959 and 1960, many boats were converted to purse seining as a result of the development of nylon nets, the power-block, and improved refrigeration (Orange and Broadhead 1959). These boats were no longer dependent upon bait, but they tended to fish the same waters as baitboats. Offshore expansion was not established until 1969.

When the first quotas were imposed, it was recognized that there might be more fish available in other areas, so the increase in CPUE that was observed was not totally unexpected. Models fitted to data from previous years were inapplicable to the new regime, so they were refitted, omitting the data for earlier years, and the estimated maximum sustainable yield approximately doubled.

Some Analytic Preliminaries

Before modelling the yellowfin population specifically, it is useful to look at how the general equation for a population with logistic growth and constant dispersion might be normalized. This will show how the number of parameters could be reduced, and it might increase our understanding of the effects and interactions of various parameters. The model given below was introduced by Fisher (1937), who used it to investigate the spatial propagation of an advantageous gene through a population. The following normalization is described in Murray (1989):

$$(1)\quad \frac{dA}{dt} = rA\left(1-\frac{A}{K}\right) - D\frac{\partial^2 A}{\partial x^2}$$

where A represents the population at a point x, r is the intrinsic growth rate, K is the carrying capacity, and D is the coefficient of diffusion, or diffusivity. Let $A' = A/K$; then

$$\frac{dA'}{dt} = \frac{1}{K}\frac{dA}{dt}$$

and

$$\frac{\partial^2 A'}{\partial x^2} = \frac{\partial}{\partial x}\left(\frac{\partial A'}{\partial x}\right) = \frac{1}{K}\frac{\partial^2 A}{\partial x^2},$$

so A' can be substituted into equation (1):

$$\frac{dA'}{dt} = rA'(1-A') - D\frac{\partial^2 A'}{\partial x^2}.$$

At steady state, $dA/dt = 0$ and

$$\frac{\partial^2 A'}{\partial x^2} = \frac{r}{D}A'(1-A').$$

Substituting $z = \sqrt{(r/D)}x$ for x:

$$\frac{\partial^2 A'}{\partial z^2} = \frac{\partial^2 A'}{\partial x^2}\left(\frac{\partial x}{\partial z}\right)^2 = A'(1-A').$$

This completes the normalization for the steady-state case. Dividing through by K standardises the amplitude of this class of functions. A/K characterises the saturation of the locality. Dividing distance coordinates by $\sqrt{(D/r)}$ scales distance with respect to diffusion and growth; $\sqrt{(D/r)}$ is known as the step-length.

It is not surprising that if K is spatially uniform, then changes in its value have no effect on the *shape* of the population distribution at steady state. That is determined by the ratio of D to r. Doubling the intrinsic growth rate of the organism will have no effect on abundance anywhere

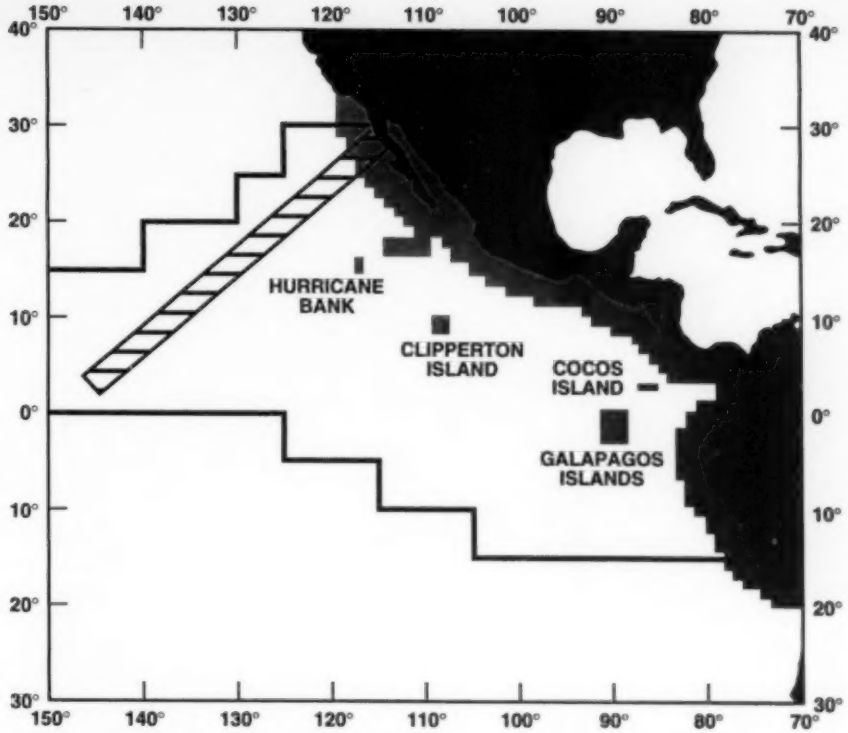

FIG. 1. Area in which fishing took place before the offshore expansion of the late 1960's, defined by the shaded area. The model described in the text represents a transect (hatched area) through the range of the fish estimated by the limits of the current fishery (bold lines).

if the rate of diffusion is also doubled. Why this is so may not be immediately obvious, but it is intuitive if one notes that at steady state the in situ growth equals the net diffusive transport everywhere but at the coastal boundary. Growth is directly proportional to r, as the transport is to D, so the steady-state distribution remains invariant while the ratio of the two is unchanged.

A nonuniform steady state can be maintained only by a boundary flux, which is identified with catch to the fishery. The catch, removed at the boundary, equals the sum of production throughout the transect. At any point, production equals $rKA'(1 - A')$. If C is the catch, then

$$(2)\quad C = qEA_0 = rK\int_0^L A'_x\,(1 - A'_x)\,dx$$

where q is the coefficient of catchability, A_0 is the abundance in the boundary cell, and E is the effort expended there.

The spatial distribution of A' is determined by the boundary condition, the length of the transect, L, and the step-length, $\sqrt{(D/r)}$. For any transect of sufficient length, and any given boundary condition, the distribution of abundance remains constant after scaling by $\sqrt{(D/r)}$. Scaling the length has the effect of multiplying the distance over which production occurs, so the catch is scaled by the same factor. "Sufficient length" refers to a requirement that the abundance at the far-shore limit of the transect be practically unaffected by the fishery, that A_L does not fall significantly below K. This allows the transect to be treated as semiinfinite — unbounded in the far-shore direction.

Equation (2) can be extended to two dimensions to model a fishery confined to an isolated island:

$$C = qEA_0 = rK\int_0^L A'_x\,(1 - A'_x)\,2\pi x\,dx$$

but the distribution A' will be different from the 1D case. Instead of equation (1), the following (Crank 1986) is more appropriate:

$$\frac{dA}{dt} = r\,A\left(1 - \frac{A}{K}\right) - \frac{1}{r}\,\frac{\partial}{\partial x}\left(rD\,\frac{\partial A}{\partial x}\right).$$

Spatial Simulation Model

The model described by equation (1) is unidimensional; abundance is assumed identical in areas immediately adjacent to the transect, so orthogonal movement can be ignored. A single transect, normal to the coast, is envisaged (Fig. 1). The figure suggests that the range of the baitboats and purse seiners of the early period was limited to approximately 120 nautical miles (NM) from shore. This is the level of spatial discrimination used in the model; the transect is approximately 2400 NM long and is represented by 20 cells, each 120 NM long.

The parameters of the production function embedded in equation (1) were estimated using the standard aggregate model (IATTC 1992). The estimated maximum sustainable yield was 328 000 tons for the eastern Pacific Ocean, corresponding to an r of 1.6·yr^{-1} and a carrying capacity of

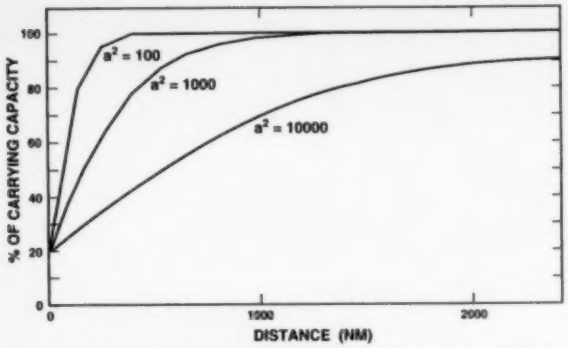

FIG. 2. Effect of a^2 on the steady-state distribution when the population is exploited only along the coast. r = 1.6. The carrying capacity was constant throughout the transect. The normalized form shown here is independent of any particular value for K.

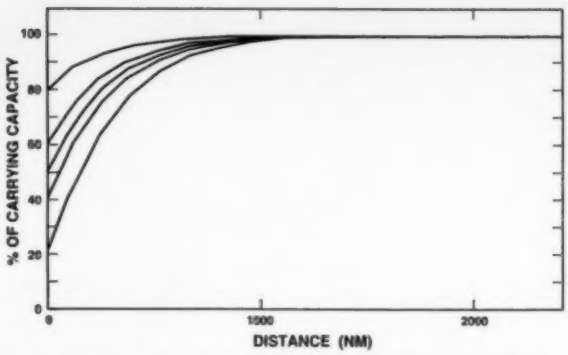

FIG. 3. Effect of various levels of coastal exploitation on the steady-state distribution. a^2 = 1000 NM^2, r = 1.6, K = 1000 tons·cell^{-1}. The abundance at the coastal region was constrained to 20, 40, 60, and 80% of its carrying capacity to reflect different rates of exploitation.

820 000 tons. The range of the fishery is illustrated in Fig. 1; it covers approximately 7 million square nautical miles. Each cell is assumed to be square, so it represents ($120^2/7 \times 10^6$) of the fishery's area. One thousand tons was assigned as the approximate carrying capacity for each cell of the model.

Bayliff (1979, 1984) determined the mean squared dispersion, a^2, to vary widely, but with 1000 NM^2·d^{-1} as a typical value, which was used to obtain a value for D: $D = a^2/4$. D was then scaled for area by dividing by the square of the interval size.

Fishing was assumed to occur only within the first cell of the model. It was not modelled explicitly, but was considered the rationale for imposing a boundary condition; the population was depressed below the carrying capacity within this interval. The flux from the boundary necessary to maintain the population below its carrying capacity represented the catch of the fishery and consisted of the sum of local production and diffusive transport to that cell. A zero flux condition was imposed at the opposite boundary of the transect.

The initial conditions in every case were such that the population was at its carrying capacity everywhere but within the cell that represented the coastal boundary, where it was held at a specified reduced level. With fewer fish in that cell, the diffusive flow from it is reduced, and hence a net flow is induced towards the coastal boundary. Production is stimulated in each cell as the biomass drops below carrying capacity. Stability is regained when the local production in each cell is equal to the net flow from it. There is a net drift of biomass towards the coast throughout the transect.

The criterion used for stopping the iterative model was that local production should equal the diffusive loss from the cell. Convergence was rapid: values were within 10% of their limiting values in one year of simulated time and within 2% in three simulated years.

Analysis has already given some insight to how the parameters interact to determine the spatial distribution, diffusive transport, and catch of fish. As a result, fewer runs of the simulation are necessary.

Results

Figure 2 shows the distribution of fish that results when the boundary condition is fixed at 20% of the carrying capacity. The nominal value for a^2 (a^2 = 4D) of 1000 NM^2·d^{-1} was perturbed by an order of magnitude in each direction to indicate the sensitivity of the distribution to this parameter. The effect of the fishery was undetectable in the far-shore section of the model except when a^2 was 10 000 NM^2·d^{-1}. The assumption used in the preliminary analytical section can be considered reasonable for the other values of a^2 used. Diminishing r by a factor of 10 while increasing a^2 by the same proportion, and vice versa, had no effect on the distribution of abundance; this confirmed the preliminary analysis. It was unnecessary to investigate changes in r any further — reducing r has the same effect on distribution as increasing a^2, and catch varies as $\sqrt{r}$ and $1/a$.

The effect of varying the boundary condition can be seen in Fig. 3. Maintaining the boundary condition requires that fish be removed (caught) from the end-cell. The smaller the population there, the steeper the gradient in abundance, and the further offshore the population is affected.

Depression of the population at the boundary enhances local production until the population goes below half of its local carrying capacity — then, local production declines. The reduction in local production is, however, generally less than the enhanced diffusive transport of fish to the coastal area which derives from the increased gradient.

To calculate the fishing effort that would be sustained by maintaining those low levels in the coastal area, the catch equation is used: $C = qEA$ where C is the catch, q is the coefficient of catchability, E is the effort, and A is the abundance within the local area. The value of q comes from the same source as the values for r and K. For this study, K has been divided to represent the smaller area of the each cell, and q must be multiplied by the same factor. This modified value of q (q = 0.0156) represents the conditional probability that a single unit of effort will catch a particular fish *given that fish lies in the same area where the effort is expended*. The total effort required to maintain the equilibrium is simply C/qA where the catch equals the sum of the local production and net immigration, which at equilibrium is the sum, or integral, of production over the rest of the model. The results of these calculations can be seen in Fig. 4, which gives the effort necessary to maintain the coastal abundance at any level. Finally, the same information can be reoriented to give the traditional yield versus effort

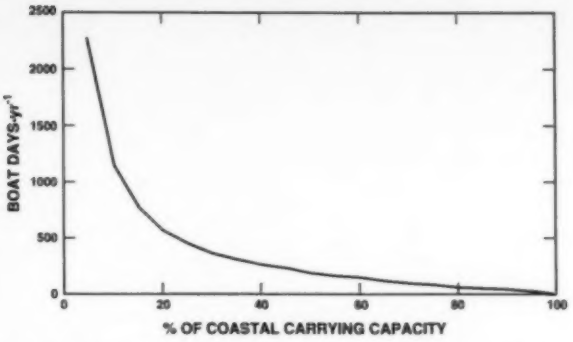

FIG. 4. Effort sustainable (boat days·yr^{-1}) as a function of standing stock of biomass (percentage of carrying capacity) within the coastal region, given that direct exploitation occurs only at the coast. a^2 = 1000 NM^2·d^{-1}, r = 1.6, K = 1000 tons·cell^{-1}.

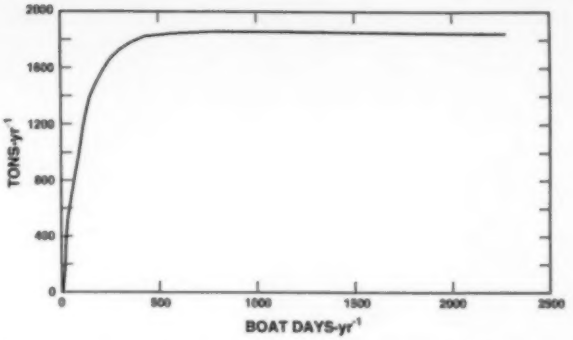

FIG. 5. Yield (tons·yr^{-1}) versus effort (boat days·yr^{-1}) at steady state for a coastal fishery. a^2 = 1000 NM^2·d^{-1}, r = 1.6, K = 1000 tons·cell^{-1}.

curve shown in Fig. 5. Note that all these results are for equilibrium conditions.

Discussion

Clearly, asymmetry is to be expected in the yield curve when catches are taken from a small part of the range of the fish and depend upon diffusive migration to the zone of exploitation. The asymmetry reported by Pella and Tomlinson (1969) for yellowfin could have arisen from the pattern of exploitation, rather than from intrinsic biology. Hilborn and Walters (1992) argued that there is rarely sufficient information to determine the extra parameter used in the Pella and Tomlinson model. In the model described here, the asymmetry is primarily controlled by the rate of diffusion, but it would be confounded with the range of fish, the size of the zone fished, and the intrinsic rate of population growth, if these were not assumed. If the diffusion parameter is known and a rough estimate of the potential range of the fish is available, then the problem reduces to fitting just two parameters, as for the Schaefer model.

Greater diffusion coefficients increase the transport of fish towards the zone of exploitation and lead to a higher CPUE for a given level of effort. The gradient in abundance decreases and the "fetch" of the fishery, the distance from the zone of exploitation at which abundance is affected, is extended. Production is stimulated in a greater portion of the fish's range, and sustainable yield will increase with diffusivity until the entire range is overexploited. Figure 3 shows, for the given estimate of diffusivity, that the fetch of the fishery is less than the range of the fishery, and the abundance in the far-shore portion is only slightly depressed when dispersion is increased by an order of magnitude.

If one were to introduce the simplest economic theory to this model, assuming a fixed price of fish and cost of effort, then the geographic restriction of the fishery would remain unexplained. The costs of operation are, however, unlikely to be fixed. Fishing far from shore increases transit time, which takes labour, fuel, etc., and remoteness may add risk, which can also be assigned a cost. If costs of operation increase with distance from shore, then the fishery should be expected to extend to the point where the additional costs equal the extra revenue gained from increased catches. The distribution of fish evolves under fishing pressure. The rate of diffusion of the population will interact with the cost function of the industry to determine the development of the fishery. A small a^2 implies low immigration into fished areas, and the consequent steep increase in abundance just beyond the current range of the fishing fleet will encourage rapid geographic expansion unless costs increase more with distance than do catch rates. Similarly, larger values of a^2 lead to less incentive to pioneer new areas, but rapid expansion could still occur if the cost function is relatively flat.

There was a discontinuity in the expansion of the eastern Pacific yellowfin fishery: a gradual expansion occurred from its inception, and then a dramatic increase in the area fished occurred in the late 1960's. In this case, regulation may have played a role, inducing technological change, which altered the cost of operation as a function of distance from shore.

Conservation restrictions were first imposed in 1966, and this coincided with an unprecedented bout of boat building. The new boats were much larger than those constructed previously, and older boats were returned to yards to be "stretched" — they were cut in two, and a new hull section was welded in place. The larger boats could catch more fish, which extended the average time at sea and reduced transit time as a fraction of each trip. They were also more able to risk poor weather far from shore. These two factors altered the cost of effort as a function of distance from shore, reducing its increase with distance. But this was not the reason for the increase in size of vessels. The fleet changed quickly from the year that regulations were imposed, while offshore fishing was not firmly established until 3 yr later (IATTC 1970). Regulation of the fishery was in the form of an annual catch quota agreed by the commissioners of the IATTC which allowed each boat to compete by attempting to maximise its portion of that quota. The staff of the IATTC had the task of determining the date at which the fishery would be "closed". After closure, any boat at sea was allowed to finish its trip, and any that were in port were allowed a "free trip", that is, they were allowed to put to sea and make another trip. (There were special allocations for some boats of developing countries, but these did not land a substantial portion of the catch (for details see Peterson and Bayliff (1985).) The fleet was closely monitored and the extra catch anticipated in the declaration of closure. These regulations favoured the larger boats, which were able to take more fish after the date of closure.

The economic argument concerning the development of the fishery has been developed informally because it is simple and because formal symbolism would add nothing to the discussion. The primary model presented here, which is simply a spatial form of the Schaefer model with diffusing fish, is proposed mainly for its heuristic value. It suggests a high degree of asymmetry for the yield–effort curve, with only a slight decline in yield beyond maximum sustainable yield, whereas Pella and Tomlinson (1969) reported that the data suggest a more significant decline. This discrepancy is unlikely to be simply the result of the parameterization. Varying carrying capacity would not change the shape of the yield curve, only its values. If the coastal section of exploitation were smaller, then the yield would have been a monotonically increasing function of effort. Given that the coastal zone is fixed, less asymmetry would be displayed if the population range were smaller, diffusion greater, or the intrinsic growth rate less, that is, if the range contained fewer step-lengths, $\sqrt{(D/r)}$. Such circumstances would render the unfished zone less of a refuge for the population.

The range of the fish does not everywhere extend to the length of the transect, but Fig. 2 and 3 indicate that a considerable reduction in range would be necessary to enable fishing to substantially affect abundance in the most distant part. Likewise, changing the diffusion coefficient by an order of magnitude, which reduces the step-length by a factor greater than 3, leaves abundance well above 50% in the part of the transect furthest from the exploited coastal zone. It is also unlikely that the intrinsic rate of growth could be overestimated by a factor greater than 3, and even that error would be insufficient to greatly affect the shape of the yield curve.

The model has been evaluated only for equilibrium conditions. The implementation as described here, using parameters for yellowfin tuna in the eastern Pacific Ocean, takes between 1 and 3 yr to converge to steady state. Alverson (1960 and 1963) showed the seasonal and spatial pattern of fishing during the 1950's and the early 1960's. There was a gradual increase in overall fishing effort throughout this period, but the pace was slow enough that the model described would be able to track the equilibrium. Seasonal variability in the spatial pattern could be serious, but effort was remarkably uniform throughout the year, with the exception that effort did not occur during winter and spring at the northern and southern extremes of the fishery.

If the discrepancy between the model and observation is not due to the particular parameterization, or to the assumption of equilibrium, then it may be due to the structure. The environment is assumed uniform, which leads to the suggestion that the majority of fish taken by the coastal fishery were transported by diffusion, with relatively little local production of biomass. This is probably untrue. Although the fishery now covers the entire range of the fish, the coastal zone still yields a disproportionate fraction of the catch. Primary production is greater inshore (Berger 1989), and Mullen (1989) argued that the distribution of yellowfin reflects that of primary productivity through modification of the diffusion coefficient. If this is so, then catches would have been greater due to increased local production and enhanced diffusive transport. The model does indeed underestimate the catches that can be obtained from a coastal fishery. If one scales by length of coastline, the maximum yield suggested is less than half of that estimated by Pella and Tomlinson (1969). This model is not presented as a definitive exemplar that could be used for management, but rather as a paradigm for understanding when a simpler model, the aggregate Schaefer model, should be viewed with caution.

The errors stemming from assuming homogeneity are clearest when fishing is confined to one part of the range of the fishery, but if homogeneity is assumed, errors will occur whenever either effort or abundance is not distributed uniformly. The potential importance of spatial effects for other stocks of fish whose movement is primarily diffusive can be gauged by comparing the step-length, $\sqrt{(D/r)}$, with their range. Fishing may impose spatial structure on a much smaller scale for populations of fish more sedentary than tunas.

It was shown in the preliminary analysis that extending this work to two dimensions is not trivial. The equation that determines the distribution of abundance is different for a fishery centred around an isolated island. Nevertheless, it is clear that much greater catches could be taken from an isolated island because fish would be drawn from every direction.

Conclusion

Cushing (1968) defined a stock to be a population whose reproduction, growth, and mortality are homogeneous. Thus, a stock can be a subset of a population. A localised fishery, by changing the mortality rate, induces heterogeneity unless the fish move at random and typically traverse the entire range of the population. In that case the stock consists of the entire population. On the other hand, if the fish are sedentary, then the stock consists of just that part of the population that resides in the exploited zone. A problem arises for the definition between the two extreme cases.

Yellowfin tuna in the eastern Pacific Ocean provide an example of the problem; they are highly mobile, but their range is vast. When fishing was confined to the coastal zone, catches were dependent upon immigration from offshore. The immediate neighbourhood beyond the range of the fishery was strongly affected by fishing, while remote areas were almost unaffected. Clearly the stock consisted of more than those fish that were within the zone fished, yet much of the population was unavailable to the fishery.

If the fishery is modelled assuming diffusive transport, then increasing the exploitation of the coastal zone increases the distance to which any specified effect on abundance could be detected. The rate at which these effects decline with distance is a function of the growth parameter of the population as well as the rate of diffusion. The high rate of intrinsic growth of yellowfin tempers the effect of their high rate of diffusion. This effect, and the size of their range, is such that their movement is insufficient to maintain a distribution that is nearly uniform when subject to spatially variable fishing pressure.

The errors that stem from assuming homogeneity are greatest when only part of the fishes' range is exploited, as in the eastern Pacific Ocean prior to the introduction of large purse seiners. Exploiting only along the edge of a large region limits the damage that can be done to a population by overexploitation, although catch rates may fall to uneconomic levels. The efficacy of the offshore zone as a refuge depends upon the intrinsic growth rate of the population, the mobility of the fish, and the size of both the exploited and unexploited zones.

The output of the model conforms to the form of the data reported by Pella and Tomlinson (1969), but the asymmetry is more pronounced. This might be due to the use of the simplifying assumption that the environment has the same productive capacity everywhere. Having discarded one simplification, that the population is uniformly distributed and exploitation is equal everywhere, another is exposed: the environment is not homogeneous.

The use of catch quotas and the phenomenon of the "free trip" may have been responsible for stimulating the construction of larger boats. These boats were able to spend longer periods at sea and could operate more easily far from shore, where severe weather is a greater deterrent to smaller boats. Thus the regulations may have been responsible for stimulating the expansion of the fishery, which provided the data that showed that the early estimates of maximum sustainable yield were conservative.

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An Inverse Model Analysis of Planktonic Food Webs in Experimental Lakes

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We used inverse methods to reconstruct carbon flows in experimental lakes where the fish community had been purposely altered. These analyses were applied to three years of data from a reference lake and two experimental lakes located in Gogebic County, Michigan. We reconstructed seasonally averaged flows among two size groups of phytoplankton, heterotrophic bacteria, microzooplankton, cladocerans, and copepods. The inverse analysis produced significantly different flow networks for the different lakes that agreed qualitatively with known chemical and biological differences between lakes and with other analyses of the impact of fish manipulations on food web structure and dynamics. The results pointed to alterations in grazing pressure on the phytoplankton that parallel changes in the size and abundance of cladocerans and copepods among lakes. Estimated flows through the microbial food web indicated low bacterial production efficiencies and small carbon transfers from the microbial food web to the larger zooplankton. This study demonstrates the use of inverse methods to identify and compare flow patterns across ecosystems and suggests that microbial flows are relatively insensitive to changes at the upper trophic levels.

Nous avons appliqué des méthodes inverses pour reconstruire les flux de carbone dans des lacs expérimentaux dans lesquels les communautés de poisson ont été altérées. Ces analyses ont été appliquées à trois années de données d'un lac de référence et de deux lacs expérimentaux situés dans le comté Gogebic, Michigan. Nous avons reconstruit les flux saisonniers entre deux classes de taille de phytoplancton, les bactéries hétérotrophiques, le microzooplancton, les cladocères et les copépodes. L'analyse inverse a produit des réseaux de flux entre les compartiments qui sont significativement différents entre les différents lacs et qui s'accordent qualitativement avec des différences connues dans la chimie et la biologie de ces lacs et avec d'autres analyses de l'impact des manipulations des communautés de poissons sur la structure et la dynamique des réseaux trophiques. Les résultats montrent des altérations de la pression de broutage sur le phytoplancton en parallèle avec des changements dans la taille et l'abondance des cladocères et des copépodes. Les flux estimés à travers le réseau microbien indiquent des efficacités de croissance des bactéries faibles et de petits transferts de carbone du réseau microbien au zooplancton. Cette étude démontre l'utilité des méthodes inverses pour identifier et comparer entre les écosystèmes les patrons de transferts trophiques. Nous suggérons à partir de notre analyse que les flux microbiens sont relativement insensibles aux changements aux niveaux trophiques supérieurs.

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Mass fluxes in aquatic ecosystems are related to the structure of food webs and the supply of energy and nutrients to primary producers. Numerous studies in lakes have focused on the differences in food web dynamics in communities dominated either by piscivorous or planktivorous fishes (DeMelo et al. 1992). Shifts in fish community structure may induce changes in the structure, biomass, and productivity of lower trophic levels (Carpenter et al. 1985). The changes in lower trophic levels that result from cascading trophic interactions may have either transitory or long-term effects on the dynamics of aquatic ecosystems (e.g., Leavitt et al. 1989; Carpenter et al. 1993b). Few studies of trophic cascades have considered how material fluxes in pelagic systems change with differing food web structure (but see Carpenter et al. 1992). In addition, few studies have considered how modifications in organisms at the top of the food web might impact heterotrophic microbial processes, despite considerable research indicating that these organisms account for much of the energy flow and nutrient recycling in planktonic systems (Stockner and Porter 1988; Sherr and Sherr 1991).

A crucial problem in assessing the response of aquatic systems to cascading trophic interactions is that most of the flows between food web constituents are difficult to measure. These unknown flows may be assessed by inverse modeling, which has been used extensively in the analysis of physical systems (e.g., Wunsch 1978; Bolin et al. 1983) and has recently been applied to food webs (Vézina and Platt 1988; Jackson and Eldridge 1992). Inverse modeling is closely related to multidimensional parameter optimization techniques that have been suggested or applied to estimate energy or material transfers in food webs (Halfon 1979). G. Radach (p. 48 in Platt et al. 1981) concluded that the utility of optimization techniques (which he called inverse methods) is

limited by the rarity of the long multivariate time series required to reliably estimate parameters. Vézina and Platt (1988) proposed to circumvent this difficulty by following practices in geophysical inverse methodology and directly incorporating a priori knowledge along with the data to constrain the parameter estimates. Inverse methods are formal procedures to estimate parameters of models that cannot be fully constrained by the available observations (underdetermined models).

The other novel aspect of this approach is that prior knowledge is incorporated as ranges of possibilities (i.e., a flow can take any value within a defined range) that reflect the uncertainties concerning many biological processes. The method calculates the "best" solution that falls within those ranges. This allows us in particular to circumvent the exact specification of nonlinear interactions in the food web models. We rely on ranges to bound possible excursions of the parameters. For example, if the standing stock of compartment P varied by a factor of 2 between lake A and lake B, we would expect the flows from this compartment to also change by a factor of 2 in a linear model. However, inexact constraints in a multidimensional linear system allow nonlinear differences (by a factor other than 2) between the flows estimated for lakes A and B.

In summary, the inverse techniques we apply here rely heavily on inexact constraints to compensate for sparse or missing data and to deal implicitly with nonlinear variations. Typically, the ratio of inexact constraints to "hard" data in our models is 3 to 1, reflecting the general state of knowledge for many ecological systems. Our application of inexact constraints to this problem is a significant difference from the bulk of optimization procedures that have been applied in ecology in the past, which rely more on the data and on exact constraint rules. In any case, the use of formal procedures for estimating food web parameters is not widespread and the techniques available to do so need to be demonstrated and refined.

The inverse technique is described in detail in Vézina and Platt (1988) and was modified slightly by Jackson and Eldridge (1992). The general approach is to develop a compartmental food web model and constrain the model with measured flows. Unmeasured flows are bounded by a series of constraint conditions that are derived from prior knowledge of general allometric trends, limits to production efficiencies, and the partitioning among energetic pathways (e.g., respiration and excretion). A solution for all the flows is then computed that is consistent with the data and the constraint conditions. The inverse modeling approach addresses the typical situation where direct flow measurements are much fewer than the total number of flows within a given food web.

In this paper, we combine data from whole-lake fish manipulations with a simple compartmental model of the planktonic food web to examine changes in carbon flows in response to cascading trophic interactions. Weekly measures of the standing stocks of all components of the food web as well as some of the major flows (e.g., primary and bacterial production) over three years in three different lakes provide an extensive data set for inverse model analysis. Our study has two purposes. First, we use the data sets to examine the utility of inverse modeling as a method for analyzing food webs; inverse modeling is a relatively new and untested method in ecological studies. Data from the experimental manipulations provide a variety of food web conditions. We examine whether the inverse modeling method can produce plausible flow structures that also discriminate between the major results of the ecosystem experiments. Our second interest is to assess carbon flows as revealed by the inverse model solutions. We evaluate how flows may have changed in response to the fish community manipulations. We focus on two types of flows that are particularly difficult to measure empirically: zooplankton grazing on phytoplankton and carbon uptake and loss by heterotrophic bacteria.

Study Sites and Experimental Manipulations

The experimental studies on which the model analysis is based were conducted in Peter and Tuesday lakes located in Gogebic County, Michigan. A third lake, Paul, served as a reference system. These lakes, the experimental manipulations conducted, and the results from these studies have been previously described (Carpenter and Kitchell 1993). Here, we only briefly sketch the manipulations and major responses of the lakes for the years 1988–90. All data used to develop the model were based on weekly samples taken during the summer stratified season.

The experiment in Tuesday Lake involved observing the recovery of planktivorous minnow populations after the removal of largemouth bass (*Micropterus salmoides*). Minnow populations (principally the northern redbelly dace (*Phoxinus eos*)) underwent exponential growth over the period 1988–90 (Hodgson et al. 1993). In response to the increase in planktivory from minnows, the zooplankton community structure shifted abruptly at the end of 1988 when the large-bodied species of *Daphnia* declined. Subsequently, the zooplankton community was dominated by an assemblage of small species, and phytoplankton biomass and productivity increased about 30% (Carpenter et al. 1993b).

The experiment in Peter Lake was designed to simulate the effects of piscivore removal on a system containing both planktivores and piscivores (Carpenter and Kitchell 1993). Planktivores were added to the lake each year in the spring and piscivore populations were reduced. In 1988 and 1989, 3000 fingerling rainbow trout (*Oncorhynchus mykiss*) were introduced and 2000 golden shiner (*Notemigonus crysoleucas*) were introduced in 1990 (Hodgson et al. 1993). Piscivores, including largemouth bass and rainbow trout (age 1+), were almost completely removed in 1989 and 1990 by a combination of electroshocking, angling, and gillnetting (Hodgson et al. 1993). This experiment resulted in periods of increased planktivory that varied among years and resulted in large oscillations in zooplankton size structure and biomass (Soranno et al. 1993b). Phytoplankton biomass and production were also affected by shifts in the size of cladocerans (Carpenter et al. 1993b).

Methods

Model Description

We developed a compartmental model of carbon flows for the photic zone during summer (Fig. 1). The model structure and flows represent a compromise between the model complexity required to represent the interactions of interest and the need to keep the number of flows to be estimated as small as possible. The compartments are nano- (<30 μm) and net (>30 μm) phytoplankton, heterotrophic

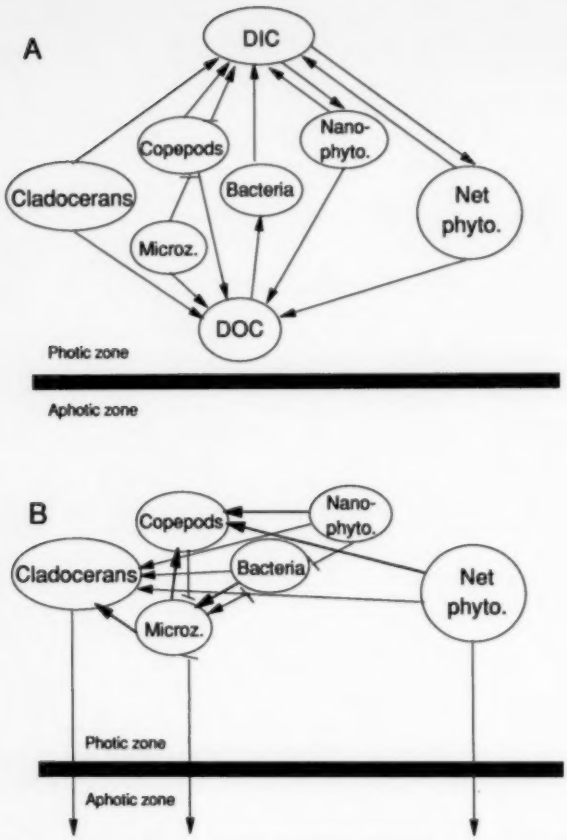

FIG. 1. Compartmental model of carbon flows in an epilimnetic food web. (A) Flows of DIC and DOC; (B) flows of particulate carbon.

bacteria, microzooplankton, cladocerans, and copepods. In the model, all living compartments add to the dissolved inorganic carbon (DIC) pool through respiration and contribute to the dissolved organic carbon (DOC) pool through excretion. We assumed that DOC release by bacteria is negligible compared with their DOC uptake.

The microzooplankton compartment represents a composite of rotifers and heterotrophic flagellates. We did not include ciliates in the microzooplankton estimates because complete data on these organisms were not available. Estimates using data from 1990 only indicate that ciliates account for 9–18% of the total microzooplankton biomass in the three lakes; excluding them from the model probably does not lead to serious underestimates of flows through the microzooplankton.

Trophic flows are limited by the prevalent feeding mode and by the relative sizes of predator and prey. In the model, cladocerans can graze on all the smaller-sized compartments, copepods are not allowed to consume bacteria, and the microzooplankton are restricted to feeding on bacteria and nanophytoplankton.

Representation of the production and fate of detrital material was highly simplified in the model. We assumed that bacteria, nanophytoplankton, and microzooplankton do not produce particulate detritus. Instead, detrital flows from these compartments move directly to the DOC pool (Fig. 1). Net phytoplankton, cladocerans, and copepods produce DOC or particulate detritus. The latter is considered to sink directly out of the photic zone without significant recycling.

Conservation of mass equations were written for each compartment:

$$(1)\qquad \sum_{\substack{i=1\\ i\neq j}}^{n} f_{ij} + z_{0j} - \sum_{\substack{k=1\\ k\neq j}}^{n} f_{jk} - y_{j0} = 0$$

where f_{ij} and f_{jk} represent intercompartmental flows (from compartment i to compartment j or from j to k) and z_{0j} and y_{j0} are, respectively, inflows and outflows across the boundaries of the food web. An external input of allochthonous DOC was introduced in the model as an additional source of heterotrophic activity (see below). Sedimentation of the detritus generated by phytoplankton and the large heterotrophs is the only net loss from the food web.

The mass flows among compartments are linear and determined strictly by the source compartment (donor controlled). Two-way interactions between source and sink compartments are not explicitly treated in the model. Top-down effects should appear as differences between the reference and treatment data sets that then affect the inverse solutions. This linear model by itself cannot simulate top-down interactions; however, comparisons among site- and time-specific solutions of the model should reveal these nonlinear interactions.

Use of Measured Flows in the Model

Primary production, bacterial production, and total respiration were measured weekly during the growing season (Carpenter et al. 1993b; Pace 1993). Seasonal averages of these measurements were included in the model with equations of the form

$$(2)\qquad p(f,z,y) = \bar{q}$$

where $\bar{q}$ is a seasonally averaged measured flow and $p(f,z,y)$ is a linear function that specifies the model flows (Fig. 1) that add up to the measured flow. Each equation was divided by the seasonal variance to insure that the weight of each measured flow in determining the global solution was inversely related to its variability.

Sedimentation losses of net phytoplankton from the photic zone for each lake and year were calculated by the formula

$$(3)\qquad f_{sed} = \frac{s \cdot P_{net}}{z_e}$$

where f_{sed} is the carbon flux sedimenting as net phytoplankton (milligrams per square metre per day), s is the average net phytoplankton sinking rate (metres per day) assumed constant for all lakes and all seasons (Baines and Pace 1994), P_{net} is the integrated carbon biomass of net phytoplankton (milligrams per square metre), and z_e is the seasonally averaged depth of the euphotic zone in each lake (metres). A constant variance of 10^4 was applied to calculated sedimentation fluxes as an indicator of the low precision of this estimate.

Use of Inequality Constraints in the Model

Vézina and Platt (1988) introduced a number of constraints for inverse modeling of aquatic food webs that are mostly size-dependent limits to energetic processes (e.g., respiration, ingestion) and limits on the efficiency of biological

TABLE 1. Constraints applied to biological flows in the inverse model. The same constraints apply to both phytoplankton compartments (nano- and net) and to the heterotrophic compartments other than bacteria. The constraint equations give the upper or lower bound to the corresponding flow in mg C·m^{-2}·d^{-1}. Variables used in the constraint equations are gross primary production (PP), net primary production (NPP), respiration (R), excretion (E), ingestion (I), degradation of dead phytoplankton to DOC (D_{ph}), DOC input from the watershed (D_{allo}), carbon biomass of the compartment (B), average individual size (W, pgC), and mean temperature of the euphotic zone (T).

Process	Compartments	Type of bound	Equation	Reference
Respiration	Phytoplankton	Lower	$0.05 \cdot PP$	Vézina and Platt 1988
Respiration	Phytoplankton	Upper	$0.30 \cdot PP$	Vézina and Platt 1988
Respiration	Heterotrophs	Lower	$0.03 \cdot I$	Fasham 1985
Respiration	Bacteria	Lower	$0.30 \cdot (\sum E + D_{ph} + D_{allo})$	Fasham 1985
Respiration	Heterotrophs	Upper	$(14 \cdot W^{-0.25} \cdot e^{(0.0693 \cdot (T-20))}) \cdot B$	Moloney and Field 1989
Respiration	Bacteria	Upper	$(1.7 \cdot W^{-0.25} \cdot e^{(0.0693 \cdot (T-20))}) \cdot B$	Moloney and Field 1989
Excretion	Phytoplankton	Lower	$0.02 \cdot NPP$	Baines and Pace 1991
Excretion	Phytoplankton	Upper	$0.55 \cdot NPP$	Baines and Pace 1991
Excretion	Heterotrophs	Lower	$0.10 \cdot I$	Vézina and Platt 1988
Excretion	Heterotrophs	Upper	R	Vézina and Platt 1988
Ingestion	Heterotrophs	Upper	$(63 \cdot W^{-0.25} \cdot e^{(0.0693 \cdot (T-20))}) \cdot B$	Moloney and Field 1989
Degradation to DOC	Phytoplankton	Lower	$0.10 \cdot (PP - R - E)$	Pace et al. 1984

TABLE 2. Limits to growth and assimilation efficiencies for the heterotrophic compartments.

	Bacteria	Microzooplankton	Copepods	Cladocerans
Growth efficiency (%)	10–60	10–40	5–40	5–40
Assimilation efficiency (%)	—	—	50–80	50–80

transformations (e.g., growth efficiency, assimilation efficiency). These constraints are expressed in the general form

$$(4) \quad h_L \le g(f,z,y) \le h_U$$

where h_L and h_U are lower and upper bounds and $g(f,z,y)$ is a linear function that specifies the combination of flows that add up to the lower and upper bounds.

We made a number of modifications to these original constraints. We updated the size-dependent constraints on respiration and ingestion with new empirical models from the literature (Table 1). The lower and upper bounds on the proportion of net primary production released as DOC were recalculated from a new literature review (Table 1). Constraints on phytoplankton respiration, DOC release by heterotrophs (Table 1), and metabolic efficiencies are basically the same as in Vézina and Platt (1988) (Table 2).

Vézina and Platt (1988) did not introduce any constraints on the specific grazing flows. As a result, some grazing flows can be set to zero by the inverse solution technique (see below), and the relative sizes of grazing flows may bear little relationship to the relative biomass of the prey compartments. We sought to correct this problem by introducing simple grazing functions based on the assumption that consumers graze in proportion to resource availability:

$$(5) \quad G_{kj} > \frac{B_k}{\sum B_i} \cdot \sum G_{ij}$$

where G_{kj} is the grazing flow from prey compartment k to consumer compartment j, B_k is the biomass of compartment k, $\sum G_{ij}$ is the total grazing by compartment j across all prey compartments, and $\sum B_i$ is the total biomass of the prey compartments. This is equivalent to assuming that the consumer compartments do not select against any prey.

Two exceptions were recognized. An unpublished study indicates that copepods filter nanophytoplankton about half as fast as net phytoplankton (H. Cyr, University of Toronto, Toronto, Ont., unpublished data). In addition, a recent study of cladoceran particle utilization (Brandelburger 1991) yields a filtration efficiency of 0.625 on bacteria relative to larger particles. Therefore, in both those cases, the grazing flows were bounded by

$$(6) \quad G_{kj} < \alpha_{kj} \cdot \sum_{i \neq k} G_{ij}$$

where α_{kj} is the ratio of the filtering rate on prey compartment k to the maximum filtering rate.

Finally, some limits on the biomass turnover (grams of carbon per gram of carbon per day) of the heterotrophic compartments were imposed. Lake- and year-specific limits on the turnover of cladocerans were calculated from birth rate data (Soranno et al. 1993a). Upper and lower bounds on the turnover rate were set at ±2 standard errors of the mean. Microzooplankton and copepod compartments were assumed to turn over at least once per 10 d and once per 20 d, respectively.

The constraints were calculated based on seasonal averages of the mean size and mean biomass of the compartments for each lake and year. The calculated means were substituted into the constraint equations (Tables 1 and 2), and these were used to obtain lake- and year-specific solutions.

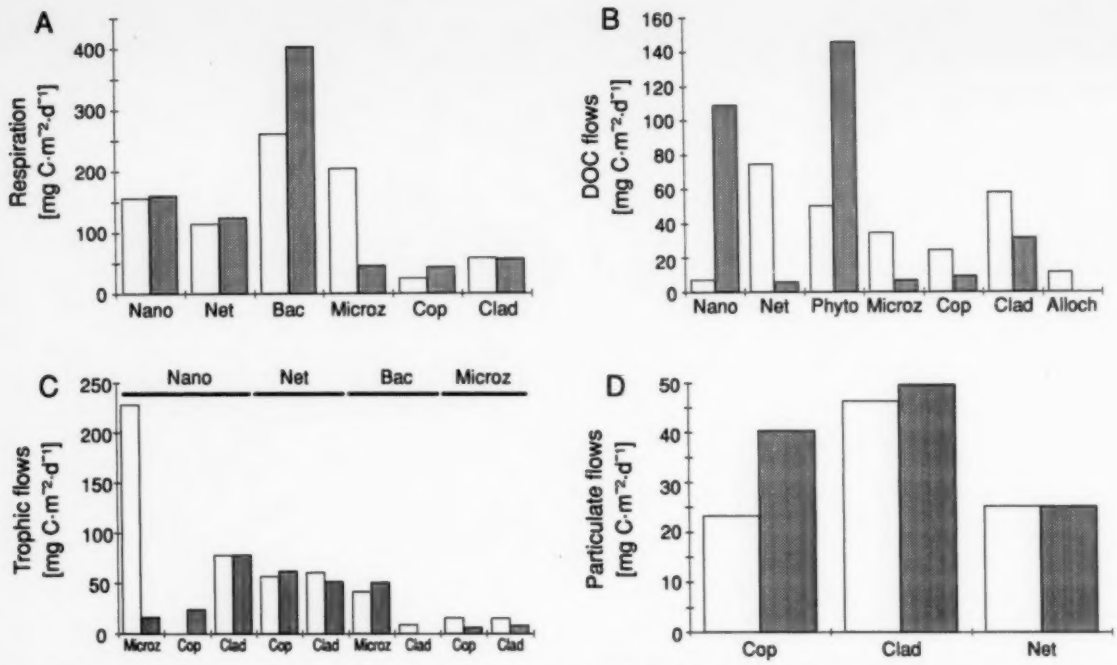

FIG. 2. Carbon flows calculated by the inverse method for Paul Lake, 1988. White bars are the estimates from the standard model and gray bars are those from the M-R model. The flows are grouped into (A) respiration flows, (B) DOC flows, (C) trophic flows, and (D) particulate detrital flows. For Fig. 2A, 2B, and 2D, the bar labels are the source compartment; for Fig. 2C, the bottom bar labels are the receiving compartments (grazers) and the top bar labels are the source compartments (prey). Abbreviations for compartments: Nano, nanophytoplankton; Net, net phytoplankton; Bac, bacteria; Microz, microzooplankton; Cop, copepods; Clad, cladocerans; Alloch, allochthonous source. Phyto in Fig. 2B refers to dissolution of detritus from both nano- and net phytoplankton.

Model Solution and Analysis

The conservation of mass (Eq. 1), flow specification (Eq. 2), and constraint (Eq. 4) equations can be collected into matrix form

$$\mathbf{Ax} = \mathbf{b} \quad (7)$$

$$\mathbf{h}_U \geq \mathbf{Gx} \geq \mathbf{h}_L \quad (8)$$

where $\mathbf{x} = [\mathbf{f}\ \mathbf{z}\ \mathbf{y}]'$, $\mathbf{b} = [\mathbf{0}\ \bar{\mathbf{q}}]'$, prime means matrix transpose, and **A** and **G** are matrices of constant coefficients. A priori weights on the conservation of mass and measured flows (**b**) and on the flows to be estimated (**x**) can be introduced as follows:

$$\mathbf{S}^{-1/2}\mathbf{AW}^{1/2}\mathbf{W}^{-1/2}\mathbf{x} = \mathbf{S}^{-1/2}\mathbf{b} \quad (9)$$

$$\mathbf{GW}^{1/2}\mathbf{W}^{-1/2}\mathbf{x} \geq \mathbf{h} \quad (10)$$

where **S** and **W** are diagonal matrices that contain weights for **b** and **x**, respectively. The diagonal of **S** has the form $[\mathbf{1}\ \sigma^2]'$ where σ^2 are the variances of the measured flows. The diagonal of **W** contains weights that control the relative importance of the flows based on a priori expectations. We used a biomass-weighted scheme, where the flows are assumed proportional to the biomass of the compartments ($w_i = B_i$). The effect of these weights is to control the relative importance of the different compartments in determining the flow structure.

Equations 9 and 10 can be solved for **x** by quadratic programming (Lawson and Hanson 1974). The solution obtained minimizes the L2 norms of the residuals ($\mathbf{Ax} - \mathbf{b}$) and of the flows (**x**), that is, it minimizes the squared errors of the measured flows and the sum of the squared flows. Equations 9 and 10 together specify over 60 constraints on 27 parameters. These constraints are not necessarily compatible and there may not be feasible solutions. We have systematically modified some of the constraints either to allow feasible solutions to be computed or to explore alternative solutions to the mass balance problem. These modifications are described in the next section.

Results

Initial Model Solutions

We first describe some general features of the inverse solutions using the flow network for Paul Lake, 1988 (Fig. 2). Gross primary production is split about equally between nano- and net phytoplankton. Respiration by the phytoplankton is high, constituting about 20–30% of gross primary production. Bacterial respiration is the most important respiration flow, followed by microzooplankton respiration; contributions of large grazers to total respiration are small. Except for nanophytoplankton, DOC flows from the biota (hereafter, recycled DOC) are spread relatively uniformly among the various compartments. Allochthonous DOC inputs make a small contribution to bacterial energy demand. Consumption of nanophytoplankton by microzooplankton is a major grazing pathway whereas the flow from nanophytoplankton to copepods is zero. Cladoceran grazing is split about equally between nano- and net

phytoplankton and is an important loss term for both compartments. Microzooplankton dominate bacterial grazing, and the utilization of microzooplankton by cladocerans and copepods are minor trophic pathways. Cladocerans are an important source of sinking detrital material; the direct contribution of autotrophic material to sinking fluxes is smaller than material produced by zooplankton.

This solution concentrated the grazing of nanophytoplankton and bacteria through the microzooplankton. We compared microzooplankton filtering rates estimated from the inverse solution with filtering rates measured in the field (Vaqué and Pace 1992) and found that the inverse estimates were on the order of 10 times higher than the field measurements. The estimated filtering rates for the other grazers were consistent with rates expected in the field (Peters and Downing 1984). This led us to limit the grazing activity of microzooplankton by restricting the size range of particles available to <5 μm for microflagellates and <20 μm for rotifers. The effect of this restriction on the inverse solution (microzooplankton restricted, or M-R hereafter) is shown in Fig. 2 for Paul Lake, 1988. Respiration flows through the bacteria were increased at the expense of microzooplankton, whose contribution to total respiration became comparable with that of the large grazers. DOC flow was routed mostly through the phytoplankton, especially through dissolution of phytoplankton detritus, and contributions by heterotrophs became much smaller. Nanophytoplankton grazing by microzooplankton fell by an order of magnitude whereas the other trophic flows remained basically unchanged. Finally, sinking flows through the large grazers were increased. The overall impact of this model change was that phytoplankton excretion losses and increased defecation by crustaceans compensated for the smaller loss term through microzooplankton.

The M-R model gave microzooplankton filtering rates on nanophytoplankton that agreed with the field estimates; however, the microzooplankton filtering rates on bacteria remained high. We tried decreasing this rate by forcing bacterial flows through the cladocerans with empirical estimates of bacterial grazing by cladocerans (Porter et al. 1983). This version of the model, however, did not work for cases where the cladoceran densities were quite high (Paul Lake, 1989, and Peter Lake, 1989 and 1990) and, in the other cases, only reduced microzooplankton filtering rates on bacteria by a factor of 2 instead of the factor of 10 needed to reconcile inverse and field estimates. Therefore, we kept the M-R model for comparison with the initial (standard) model. We recognized that the M-R model did not yield completely accurate trophic flows through the microzooplankton, but we considered it as the best model given existing information.

Consistency of Inverse Flow Networks

We computed solutions based on the standard and M-R models for the nine lake–year data sets. Feasible solutions (i.e., compatible with all the constraints) were obtained in all but one case: the M-R model for Paul Lake, 1990. To determine if the solutions were consistent and discriminated among the nine data sets, we used a principal component analysis of all the flows from the all the solutions (27 flows × 17 solutions). The first two components accounted for 46% of the total variability in flows among models and data sets and were used to map the solutions

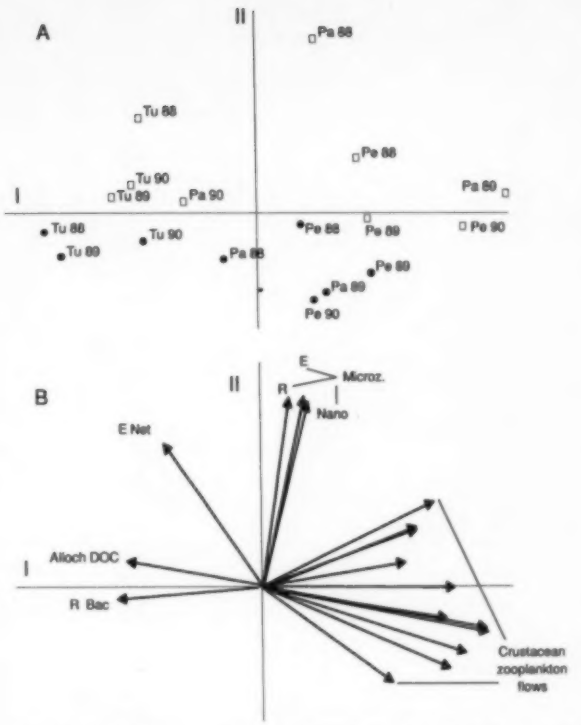

FIG. 3. (A) Positions of multidimensional inverse solutions (27 flows) on the plane formed by the first and second principal components and (B) positions of the flows that weigh most heavily on these components. Squares represent the solutions of the standard model and circles are the solutions of the M-R model. Abbreviations in Fig. 3A: Tu, Tuesday Lake; Pa, Paul Lake; Pe, Peter Lake; solutions for 1988, 1989, and 1990 are shown. Abbreviations for flows in Fig. 3B: *E*, excretion; *R*, respiration; compartments are identified as in Fig. 2. Crustacean zooplankton flows are not identified individually. Flows tend to increase in the direction of the arrows.

and the flows that were most strongly correlated with the variations among solutions.

The inverse solutions for the experimental lakes, Tuesday and Peter, map in different parts of the reduced space formed by the first two components. The solutions for Paul Lake tend to be more dispersed, as might be expected for the reference lake. Solutions for Peter Lake are found on the positive side of the first principal component (Fig. 3A), reflecting higher flows through the large consumers (Fig. 3B). Solutions for Tuesday Lake fall on the negative side of the first principal component (Fig. 3A), toward higher bacteria respiration, allochthonous DOC inputs, and phytoplankton excretion flows (Fig. 3B). The first component is largely driven by changes in the grazing and metabolic flows of the crustacean zooplankton compartments; except for bacterial respiration, the microbial flows are not strongly involved in the differences in flow patterns.

The standard solutions for Paul and Tuesday lakes fall on the positive side of the second component whereas the M-R solutions for these lakes fall on the negative side (Fig. 3A). The positive side of component 2 is related to increases in flows through the microzooplankton (Fig. 3B).

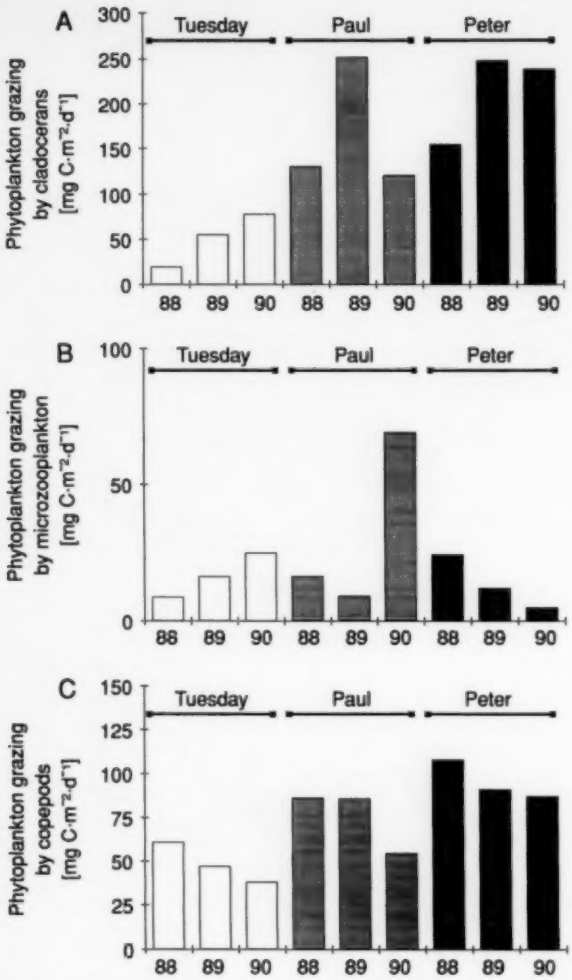

FIG. 4. Phytoplankton grazing rates inferred by the inverse models.

This generalizes the tendency, already noted for Paul Lake, 1988, of the standard model to route the flows preferentially through the microzooplankton.

Analysis of Flows

Using the microzooplankton restriction model, we analyzed the flows of carbon through the food webs for all lakes and years except for Paul Lake, 1990, where the standard model was used. We focused on the phytoplankton grazing flows and on the flows through the microbial food web. These flows have not been measured directly in the experimental lakes and we feel that it is in those areas that the inverse solutions can make the strongest contribution.

Patterns of grazing on phytoplankton

One of the intentions of the experimental manipulations was to induce shifts in zooplankton community structure that would change the grazing pressure on phytoplankton. The inverse solutions provided estimates of the various grazing flows (Fig. 4) that were difficult to measure directly. We used the model flows to evaluate how grazing may have changed in response to the manipulations. In addition, we assessed the modeled grazing flows for their consistency with other data and analyses of the ecosystem responses.

In the reference system, Paul Lake, cladocerans were the most important grazers in all three years, and grazing by cladocerans was particularly high in 1989 (Fig. 4A). This was because the large cladoceran *Holopedium gibberum* was very abundant during this year; zooplankton biomass reached the highest level recorded in Paul Lake over a 7-yr period (Soranno et al. 1993a, 1993b). Microzooplankton grazing was low except during 1990 (Fig. 4B). However, only the standard model could fit the 1990 Paul Lake data and we have no evidence to support this high estimated microzooplankton grazing. Neither rotifers nor heterotrophic flagellates occurred at higher abundances and there were no significant changes in size structure of either community in 1990 relative to other years in Paul Lake.

In Peter Lake, crustacean zooplankton grazing was a large component of the total flow in the food web. For example, crustacean zooplankton grazing accounted for 53–68% of the net primary production in Peter Lake compared with 39–57% in Paul Lake and 25–33% in Tuesday Lake over the three years. Grazing by cladocerans increased in 1989 and 1990, while copepod grazing remained stable (Fig. 4A and 4C). Microzooplankton grazing was a minor flow in all three years and declined over time (Fig. 4B). These patterns agreed with large increases in both the size and biomass of the cladocerans during 1989 and 1990, but not with shifts in the biomass of copepods (Soranno et al. 1993a).

We suspect that the magnitude of the interannual changes in grazing in Peter Lake was actually higher than that portrayed in the model. This is because, for 1989 and 1990, the upper limit on the biomass turnover of the copepods and the cladocerans had to be relaxed to obtain a feasible solution. The turnover times for these compartments reached 2–3 mo in the model. The high turnover times were needed because the model could not satisfy the high zooplankton demand given the available production. This agrees with lower measured birth rates for cladocerans (1989–90) as well as with the sharp population declines observed after an initial biomass maximum.

Modeled flows for Tuesday Lake suggested that grazing by microzooplankton and cladocerans was low in 1988 and then increased in 1989 and 1990 (Fig. 4A and 4B). In general, microzooplankton were a more important component of the total grazing in Tuesday Lake than in the other lakes. Increased microzooplankton grazing in 1989 and 1990 (Fig. 4B) was consistent with increases in rotifer populations observed over this period (Soranno et al. 1993a). Cladoceran grazing was especially low in 1988 (Fig. 4A). This result seemed surprising, as cladoceran biomass was highest in 1988 and the mean size of cladocerans declined from 1988 to 1990 as planktivory from the recovering minnow populations increased (Soranno et al. 1993a). Nevertheless, this result was consistent with the allometric formulations we used in the model. The shift to smaller zooplankton in Tuesday Lake led to a higher metabolic demand by the zooplankton in 1989 and 1990. This resulted in a solution that partitioned more of the phytoplankton carbon through the cladoceran compartment.

Microbial flows

The changes induced by the experimental manipulations offer the opportunity to analyze the sensitivity of microbial

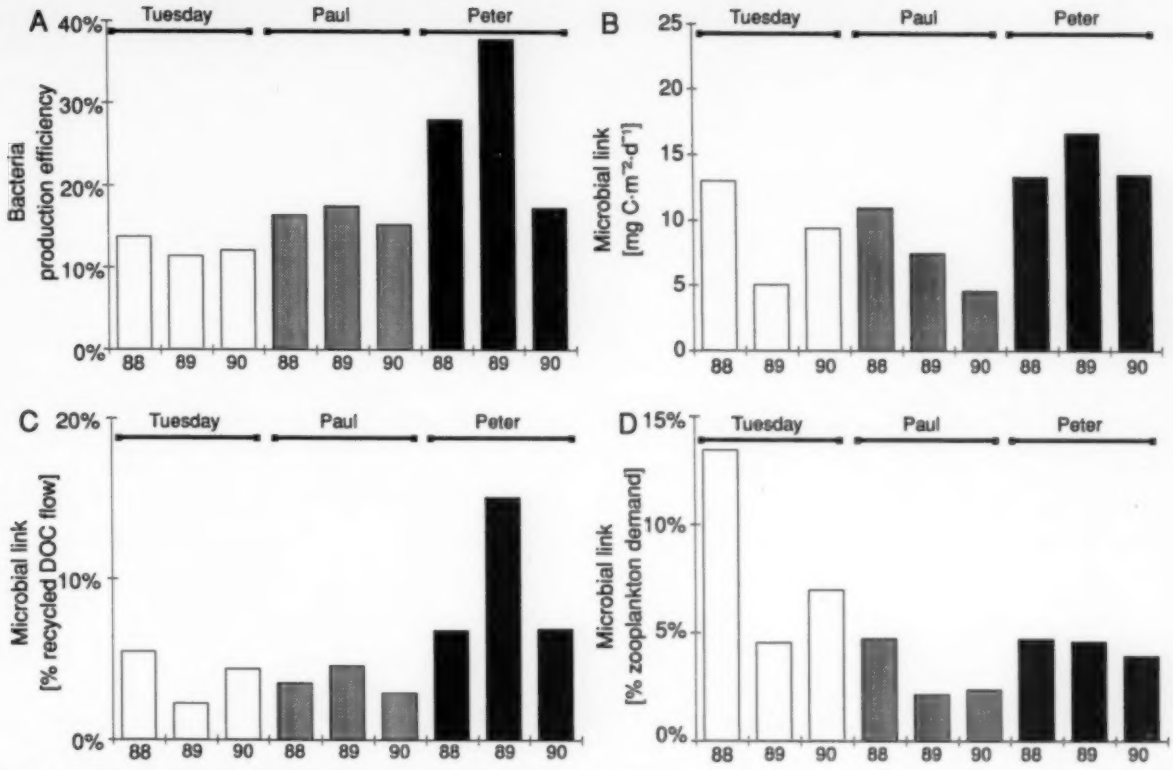

FIG. 5. (A) Bacterial production efficiency and (B–D) flows of particulate carbon from the microbial food web (bacteria and microzooplankton) to the zooplankton (copepods and cladocerans) in carbon units (Fig. 5B) and as a proportion of the gross DOC flow (Fig. 5C) and of the zooplankton carbon demand (Fig. 5D).

flows to shifts in food web structure. From the flow networks, we derived bacterial production efficiency, the amount of bacterial carbon transferred to crustacean zooplankton, and the ratios of this carbon transfer to DOC flow and zooplankton demand (Fig. 5). In the model, bacterial production efficiency was the ratio of predatory consumption to carbon input to this compartment. This efficiency was about 10–15% in Tuesday and Paul lakes and 20–40% in Peter Lake (Fig. 5A). The low production efficiencies in Tuesday and Paul lakes resulted from the relationship between measured rates of bacterial production and total community respiration. Bacterial production was low relative to primary production, while community respiration often exceeded net primary production (Pace 1993). As a consequence, bacterial respiration in model solutions was a major flow (Fig. 2) leading to low production efficiencies. In Peter Lake, the primary production to respiration ratio exceeded 1, so less material was funnelled through bacterial respiration in the model solutions. Production efficiencies were thereby higher, although not as high as the values of 50% often used as standards in modeling studies (e.g., Pace et al. 1984; Fasham et al. 1990).

The "microbial link" is the transfer of microbial carbon to crustacean zooplankton, either by direct consumption or indirectly through the microzooplankton compartment (Fig. 5B). In absolute terms, this quantity varied from about 5 to 15 mg $C \cdot m^{-2} \cdot d^{-1}$ in the different solutions (Fig. 5B). This quantity represented a small percentage of the flow of DOC derived from biotic compartments (=recycled DOC in Fig. 5C), meaning that most of the DOC produced by constituents of the food web was respired within the microbial food web (bacteria and microzooplankton). The microbial link was also a small fraction of the crustacean zooplankton carbon demand. Mean efficiencies for the different lakes and years are all <15% and are usually around 5%, indicating that only a small component of the carbon required by zooplankton was ultimately derived from bacterial production (Fig. 5D).

According to model solutions, flows through the microbial compartments were not strongly impacted by the food web shifts. For example, despite the large changes in zooplankton biomass in Peter Lake, there was little change in the quantity of microbial production moving to larger zooplankton (Fig. 5B and 5D). Similarly, in Tuesday Lake, there was little change in the modeled flows through the microbial components even though primary production, chlorophyll, and zooplankton size structure changed over the 3-yr period.

Discussion

Evaluation of Inverse Method Results

One initial goal of our investigation was to evaluate the ability of the inverse method to discriminate among different experimental situations. For Tuesday and Peter lakes, the differences were clear and were as expected from their

contrasting trophic structures. Large grazers were generally dominant in Peter Lake and the inverse solutions produced high grazing flows through the crustacean zooplankton. Small grazers were relatively more abundant in Tuesday Lake and more of the production moved through detritus and DOC to bacteria. The inverse solutions also suggested higher allochthonous DOC utilization in Tuesday Lake than in Peter Lake. This was an original result of the inverse analysis (there were no data or constraints on this flow) and it agreed qualitatively with the trophic status of Tuesday (dystrophic) and Peter (oligotrophic) lakes. In general, the inverse analysis discriminated flow networks between the experimentally manipulated lakes that involved plausible changes in flow patterns.

The inverse analysis did not discriminate interannual differences as well as differences between the experimental lakes. In Tuesday Lake, the production, biomass, and size structure shifts induced by the minnow population recovery between 1988 and 1990 were modest (on the order of 20–30%). In Peter Lake, shifts in crustacean zooplankton were strong but transient, and their impact was difficult to resolve with a seasonally averaged model. We would expect stronger and sustained manipulation effects to appear in the flow structures estimated by inverse analysis. In any case, the M-R solutions suggested a movement toward higher crustacean grazing in both Tuesday and Peter lakes in 1989 and 1990. This change in Peter Lake can be related to the effect of the manipulation on zooplankton biomass; the unexpected increase in grazing in Tuesday Lake is discussed later.

In Paul Lake, the trophic structure was not pushed to extremes by manipulations as in Tuesday or Peter Lake. For such undisturbed situations, the model choice was more critical because the data and constraints allowed a wider range of inverse solutions. The M-R model solutions appeared as more credible descriptions of flows in the reference lake. Not only were the estimated grazing flows more realistic, but the interannuel differences in the solutions were much less pronounced than with the standard model, which was what we expected in the reference system. Nevertheless, the interannual differences in Paul Lake solutions were significant and related to significant year-to-year variations in the reference lake (e.g., the *Holopedium* bloom in 1989 and measured primary production in 1990 which was 20% below its 3-yr mean). Long-term records are needed to separate experimental effects from natural variability whatever the analysis techniques used, including inverse analysis.

Inverse models of the experimental lakes did not always produce feasible solutions. Data collated within an inverse model were not necessarily consistent with the preconceptions built into the mass balance conditions (e.g., steady state) or the constraints. The steady-state assumption was probably inappropriate for Peter Lake in 1989 and 1990. Zooplankton biomass fluctuated dramatically during the growing season and inverse solutions tracking the week-to-week dynamic changes would probably have been required to properly account for the seasonal flows. Methods to do this are under development. That feasible solutions were obtained by increasing the turnover time of crustacean zooplankton confirmed that there was a shortage of energy for large grazers in Peter Lake, in accord with observations that the artificially high zooplankton biomass was not sustainable (Soranno et al. 1993a).

Although the basic model used was linear, some of the differences in inverse solutions among lakes and years were nonlinear. For example, the proportion of the primary production grazed shifted dramatically among lakes, particularly between Tuesday Lake and both Paul and Peter lakes. There were also shifts in microbial flow patterns between Peter Lake and the other lakes. Contrary to our expectations, these shifts were more related to lake-to-lake differences than to specific experimental manipulations. Nevertheless, the inverse model was able to reveal nonlinear changes in the flow patterns implicit in the differences among data sets.

In this analysis, the interactions between nutrient and carbon flows were not considered. Phosphorus generally limits carbon production in lakes and phosphorus cycling is obviously a critical component of these food webs and of their response to manipulations. The inverse methodology can accommodate models with several materials or tracers flowing simultaneously through the system (Vézina and Platt 1988). Many of the phosphorus flows are simple stoichiometric transformations of carbon flows and do not add new information; nevertheless, the addition of cycling processes specific to phosphorus could further constrain the carbon flows. The detailed data on phosphorus cycling in these lakes were not available at the time of our analyses, but this information is now becoming available and will be used in future analyses.

Overall, the inverse analysis gave flow networks that qualitatively agreed with independent assessments of the interlake differences and of the effects of the manipulations. They also agreed with the magnitude and nature of interannual variations in the reference lake. The absence of a feasible solution could indicate either potential difficulties with the data (averaging out fluctuations), imbalances between trophic levels, or other incompatibilities between the data and the constraints. Inverse analysis proved a useful tool not only to produce flow networks, but also to verify the internal consistency of complex data sets.

Ecological Implications of Inverse Method Results

The inverse analysis provided a number of quantitative insights into flows that are not routinely measured in the field. Microbial flows and efficiencies, for example, are difficult to assess because bacterial utilization of the heterogeneous DOC pool that feeds the microbial food web is difficult to quantify in situ. The growth efficiency of bacteria in nature is uncertain (Pomeroy and Wiebe 1988) and some efficiency figure must be assumed in energy flow studies. Our inverse analysis circumvents this problem by calculating bacteria growth efficiencies that are consistent with global properties of the ecosystem. The results for these experimental lakes indicate that bacteria growth efficiencies must be low to simultaneously balance production, respiration, and the energy flows through the various trophic levels. The bacterial respiratory sink in these lakes is very high, particularly in dystrophic Tuesday Lake. Whether this result, obtained in these small lakes, can be generalized to other aquatic systems remains to be determined.

Given low bacterial efficiencies, microbial transfers to large consumers calculated by inverse modeling are small. These results are qualitatively consistent with tracer studies (Ducklow et al. 1986; Pernie et al. 1990; Wylie and Currie 1991) and with other modeling studies (Jackson and Eldridge 1992). From an ecosystem perspective, trophic links directly

from bacteria to large grazers or through micrograzers contribute little energy to the upper food web.

The efficiency of DOC utilization and transfer was highest in Peter Lake in 1989 and 1990, when there were high biomass of cladocerans and copepods. Under those conditions, the large grazers reduced the energy available to the microbial food web. The inverse models consistently calculated lower phytoplankton grazing by microzooplankton and lower DOC flows, particularly through the phytoplankton. Therefore, the inverse solutions suggested shifts from detrital to grazing pathways that may have been caused by perturbations at higher trophic levels. Nonetheless, the proportion of the zooplankton demand met by the microbial link remained negligible. Increases in microbial efficiencies did not compensate for lower absolute DOC flows or for increased zooplankton demand.

The inverse modeling also produced estimates of grazing flows that were difficult to measure in the field. The solutions gave the expected differences in grazing patterns between Tuesday and Peter lakes: relatively larger grazing flows through the microzooplankton and lower overall grazing pressure in Tuesday Lake. The only unexpected result was that overall grazing pressure in Tuesday Lake increased from 1988 to 1990 contrary to expectations based on time series analyses of cascading interactions. Specifically, the increase in phytoplankton biomass and production in Tuesday Lake between 1988 and 1990 was attributed to decreased grazing pressure by *Daphnia* (Carpenter et al. 1993a).

There are three possible explanations for this discrepancy. The first is that grazing did increase slightly as portrayed in the inverse solutions and that the increase in primary production is not a consequence of lower grazing pressure. The second is that time-averaged inverse models cannot detect the modest effect of the *Daphnia* population decline on primary production. The third possibility is that the models we used do not effectively simulate the grazing of large *Daphnia*. The standard and M-R models used in this study both predict higher metabolic demands for smaller organisms. Because they are based strictly on allometric relations, decreases in grazer size will to a certain extent compensate or even reverse the impact of decreases in grazer biomass. However, these allometric rules may not accurately portray the differences in actual feeding rates among grazers (Cyr and Pace 1992). Large *Daphnia* consume a broader spectrum of particles than most zooplankton (Burns 1968). Furthermore, while allometric models for zooplankton in general reveal that specific feeding rates decline with body size (Peters and Downing 1984), models of in situ cladoceran feeding predict that specific rates do not decline with size (Knoechel and Holtby 1986). The use of alternative formulations to parameterize this effect in inverse models, and other types of models as well, should be explored.

An important goal of ecosystem studies is to identify regular and repeatable patterns in the flows of energy and materials and to compare these flow properties within and among systems. Inverse methods were proposed as one way to move toward this objective (Vézina and Platt 1988). This study demonstrates the use of inverse methods to identify and compare flow patterns across ecosystems. From our analysis, we propose that the most fruitful directions for improvement of the inverse methodology would be (1) use of time series data to preserve dynamic information and (2) improved constraint equations to increase the ecological realism of the solutions. We believe that inverse analysis can contribute greatly to the elucidation of energy flow patterns in aquatic ecosystems that remain difficult to assess by direct observational or experimental means.

Acknowledgments

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Long-term Influence of Water Temperature, Photoperiod, and Food Deprivation on Metamorphosis of Sea Lamprey, *Petromyzon marinus*

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After 11 mo in the laboratory, significantly more sea lamprey, *Petromyzon marinus*, larvae from the Chippewa River, Michigan, metamorphosed in an ambient temperature regime (3 ± 2 animals·tank^{-1}) compared with a fixed 21°C temperature (0 animals); photoperiod and food deprivation did not have detectable effects on the incidence of metamorphosis. Metamorphosing animals in our laboratory study were significantly smaller in size (length and weight) and had a lower condition factor (CF) than animals from the same population that metamorphosed a year earlier under field and shorter term laboratory conditions. We also predicted, using criteria of 120 mm, 3.0 g, and a CF of 1.50, that 12 and 14% of the animals in the ambient and fixed temperature regimes, respectively, would metamorphose. Our prediction for the ambient temperature did not differ significantly from observed (11%). We suggest that larvae in landlocked populations of sea lamprey that are at least 120 mm long, weigh 3.0 g, and have a CF of 1.50 or greater in the fall can be predicted to metamorphose the following summer. Furthermore, our data show that low temperature during the winter followed by rising temperature in the spring is the primary environmental cue initiating metamorphosis in sea lamprey.

Après 11 mo en laboratoire, un nombre significativement plus important de larves de grande lamproie marine, *Petromyzon marinus*, de la rivière Chippewa, au Michigan, se sont métamorphosées en étant exposées à la température ambiante (3 ± 2 animaux·réservoir^{-1}) qu'à une température fixe de 21 °C (0 animaux); la photopériode et la privation de nourriture n'avaient pas d'effet détectable sur le taux de métamorphose. Les animaux qui se sont métamorphosés pendant notre étude en laboratoire étaient de taille (longueur et poids) significativement plus petite et avaient un coefficient de condition (CF) plus bas que les individus de la même population qui s'étaient métamorphosés 1 an plus tôt et qui avaient connu des conditions naturelles et un plus court séjour en laboratoire. Nous avions aussi prévu, à partir des critères de 120 mm, 3,0 g et CF = 1,50, que se métamorphoseraient 12 et 14 % des animaux dans les conditions respectives de température ambiante et de température fixe. Notre prévision pour les conditions de température ambiante ne différaient pas beaucoup des résultats obtenus (11 %). Nous postulons qu'on peut prévoir que, dans les populations de lamproie sans accès à la mer, les larves qui ont au moins une longueur de 120 mm, un poids de 3,0 g et un CF de 1,50 à l'automne se métamorphoseront l'été suivant. De plus, nos données montrent que le réchauffement printanier après les basses températures hivernales constituent le principal signal environnemental responsable du déclenchement de la métamorphose chez la grande lamproie marine.

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Larval sea lamprey, *Petromyzon marinus*, are sedentary filter-feeding fish, living in the soft substrates of streams and some lentic areas throughout the Great Lakes, the eastern coast of North America, and western Europe (Applegate 1950; Potter 1980). The growth phase of the larval period, typically lasting 3–7 yr, is followed by a metamorphosis, which leads to the development of a juvenile capable of feeding on fish (Potter 1980; Youson 1980). In the

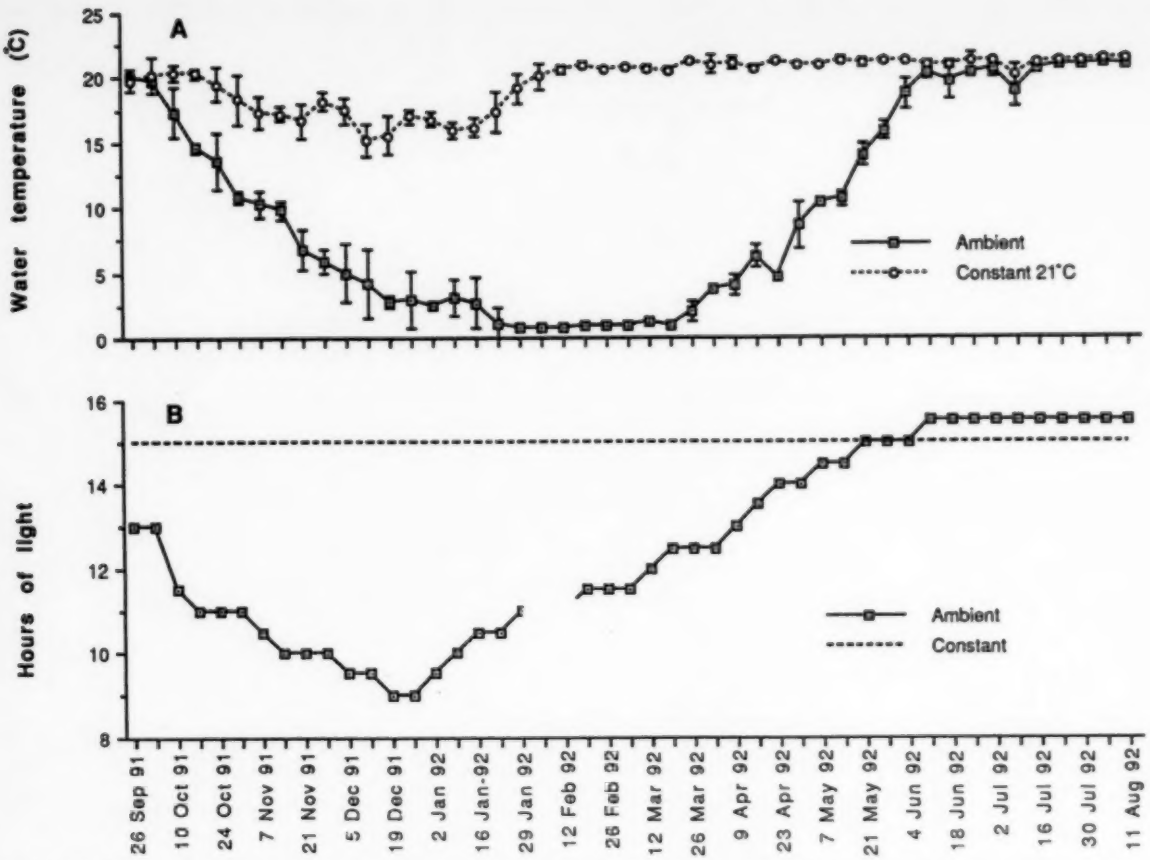

FIG. 1. (A) Water temperature and (B) photoperiod from September 1991 to August 1992. Water temperature data are weekly means ± 1 SD.

Great Lakes region, newly metamorphosed animals emerge from the substrate and migrate downstream in the fall or the following spring so that the time between the onset of metamorphosis and the first feeding on teleosts may vary from 3 to 10 mo (Applegate 1950; Potter 1980).

Metamorphosis is a nontrophic phase of the sea lamprey life cycle, during which catabolism of lipid is the primary source of energy to the animal (Lowe et al. 1973; Youson et al. 1979). Larvae may undergo a period of arrested growth prior to metamorphosis (Hardisty and Potter 1971; Potter 1980; Youson 1988), during which length does not change appreciably but lipid levels increase from about 4 to 14% of the body weight (Lowe et al. 1973; O'Boyle and Beamish 1977). This increase in lipid results in the extensive fat deposits (Youson et al. 1979), greater body weight, and higher condition factor (CF, CF = weight/length^3 × 10^6) of animals in the early stages of metamorphosis compared with larvae of metamorphosing size (≥120 mm and 3.0 g; Youson et al. 1993). The influence of starvation on the incidence of metamorphosis has never been tested in lampreys, yet starvation in larvae results in some morphological and physiological changes in the tissues and organs that resemble those seen in metamorphosing animals (Youson 1993).

One or more environmental factors are important cues initiating metamorphosis, since there is variation in the number of animals metamorphosing between streams and within a stream from year to year (Potter 1980; Purvis 1980). Studies of temperature and photoperiod have shown that the initiation of metamorphosis is sensitive to differences in water temperature (Purvis 1980; Youson et al. 1993) but not photoperiod (Cole and Youson 1981; Youson et al. 1993) in the month prior to the normal commencement of metamorphosis. However, these studies used constant environmental conditions for short periods of 1–2 mo.

Size (length and weight) and CF also affect the initiation of metamorphosis in sea lamprey. Anadromous sea lamprey enter metamorphosis only after reaching at least 110 mm and 2.0 g in size and a CF of 1.46 (Potter et al. 1978). Among landlocked sea lamprey, metamorphosing animals must be at least 120 mm and 3.0 g in size and have a CF of 1.50 or greater in the spring to enter metamorphosis the following July (Youson et al. 1993). Larvae of the appropriate size but with a CF < 1.50 will not enter metamorphosis, requiring at least 1 more yr to increase lipid levels to about 14% of their body weight (Youson et al. 1993). Thus, the length criterion identifies larvae in the arrested growth phase of the larval period, while the weight and CF criteria identify those larvae of the appropriate size with sufficient lipid reserves to enter metamorphosis.

The objective of this study was to assess the long-term

influence of photoperiod, water temperature, and food deprivation on the incidence of metamorphosis in landlocked sea lamprey. In this study, we focus on the role of progressive increases in temperature and daylength and a lack of food in the period leading up to the normal commencement of metamorphosis. We also tested the utility of 120 mm, 3.0 g, and a condition factor of 1.50 as minimum criteria for identifying presumptive metamorphosing animals in the fall prior to metamorphosis the following July.

Materials and Methods

Approximately 1000 larval sea lamprey were collected from the Chippewa River, Lake Huron, Michigan, on 19–22 August and 16–18 September 1991 and transported to the Hammond Bay Biological Station (HBBS), Michigan, where they were held in tanks at 10°C. Larvae (N = 960) having a total length ≥120 mm or a weight ≥3.0 g were randomly assigned to 32 experimental tanks (30.5 cm width × 60 cm length × 30 cm height) at a density of 30 animals·tank^{-1} (164 larvae·m^{-2}) on 19 September 1991. The tanks were supplied with aerated lake water and were placed inside cabinets with doors so that specific photoperiod regimes could be maintained during the experiment. The tanks and sand for burrowing were cleaned about once a month and the sand was replaced periodically throughout the experiment. The apparatus and other procedures were the same as those described by Youson et al. (1993).

Larval sea lamprey were exposed to two water temperature regimes (ambient, simulating the natural thermocycle in the Chippewa River, and a constant 21°C, simulating the mean summer water temperature in the Chippewa River), two photoperiod regimes (constant 15 h light : 9 h dark and the natural ambient period at the HBBS), and two levels of food availability (no food and 0.5 g baker's yeast·ind^{-1}·wk^{-1}) for 11 mo. The constant temperature and photoperiod were used to provide a comparison with previous work (see Youson et al. 1993). Ambient temperatures were adjusted weekly using data on water temperatures in the Chippewa River. Four tanks were subjected to each combination of temperature, photoperiod, and food availability. Larvae from one of eight tanks in each temperature–photoperiod combination were used to replace mortalities in experimental tanks. Replacement larvae were marked by a subcutaneous injection of fluorescent latex dye in the caudal sinus and were excluded from our data analysis.

The experiment began on 19 September 1991 and the larvae in the experimental tanks remained undisturbed, except during sand replacement, until 14–15 May 1992, when they were measured (nearest millimetre) and weighed (nearest 0.1 g). CF was calculated as

$$CF = W/L^3 \times 10^6$$

where W is weight (grams) and L is total length (millimetres). The animals were sampled (length, weight, stage of metamorphosis) on 21–22 July when the majority of metamorphosing animals should have been at stage 2 or 3 (Youson and Potter 1979). The experiment was continued until 10–11 August, when a final determination of the numbers and metamorphic stages was made and the weights and lengths were recorded. All stages of metamorphosis were determined using descriptions in Youson and Potter (1979).

The effects of temperature, photoperiod, and food availability on the incidence of metamorphosis among animals, regardless of their metamorphic stage, were analyzed using three-way ANOVA, and a priori pairwise comparisons of all main effect means were made (Sokal and Rohlf 1981). Two methods were used to predict the number of metamorphosing animals from the September data: (1) criteria of 120 mm, 3.0 g, and a CF of 1.50 (Youson et al. 1993) and (2) a 120-mm-length criterion alone. The 120-mm length is the smallest observed length of a metamorphosing sea lamprey in the Great Lakes (Potter 1980). The heterogeneity G-test of goodness-of-fit (Sokal and Rohlf 1981) was used to compare the predicted and observed number of metamorphosing animals.

Results

The mean (±SD) length, weight, and CF of larvae were 132 (9) mm, 3.23 (0.69) g, and 1.40 (0.097) at the beginning of the experiment in September 1991. There were no significant differences ($P < 0.05$) among the temperature, food, or photoperiod groups. However, at the beginning of the experiment in September the larvae were significantly (t-test, $P < 0.05$) longer, lighter, and had a lower CF than larvae from the same population at the beginning of a short-term study in June 1991, which were 129 (11) mm, 3.33 (0.87) g, and CF 1.51 (0.12) (see Youson et al. 1993). Mortality was low (three larvae, 0.3%) between September 1991 and May 1992, but mortality rose to 35 animals (3.6%) between May and July. Most of the mortality (32 larvae) was in tanks receiving 21°C water and deprived of food.

Ambient temperature reached a low of about 1°C in late January 1992 and a high of approximately 21°C by early July (Fig. 1A). Our May sampling occurred at the midpoint of the annual spring temperature rise. We maintained a fixed temperature of 21°C for 7 mo, but between mid-October 1991 and mid-February 1992, mean weekly temperature varied between 15 and 20°C. Ambient photoperiod declined from 15 h of light in September 1991 to 9 h in December and then increased to 15.5 h by June 1992 (Fig. 1B).

Effects of Environmental Factors on the Incidence of Metamorphosis

Fourty-eight of 960 animals metamorphosed and all were in the ambient temperature regime. The July and August data were consistent with respect to metamorphosis among animals receiving ambient temperature water. The majority of metamorphosing animals were at stage 1 at the end of July, and by mid-August, 48 were at stages 2 and 3 and another 14 animals were at stage 1. Our analysis of the incidence of metamorphosis is based on the stage 2 and 3 animals in August because the external signs of metamorphosis are more clearly defined at these stages than for stage 1 (Youson and Potter 1979).

In this study, the number of animals metamorphosing in a tank was affected by water temperature ($F_{1,20} = 23.8$, $P = 0.0001$) but not photoperiod ($F_{1,20} = 0.87$, $P = 0.36$) or food deprivation ($F_{1,20} = 0.00$, $P = 0.98$). There were no significant interactions among any combination of the three factors examined ($P > 0.40$). Significantly more animals in the ambient temperature regime metamorphosed than in the fixed temperature, with an average (±SD) of 3 (2.4) animals·tank^{-1} compared with 0 animals in the 21°C tanks ($P < 0.05$).

Larval sea lamprey tended to undergo negative changes in length and weight and were in poorer condition in July

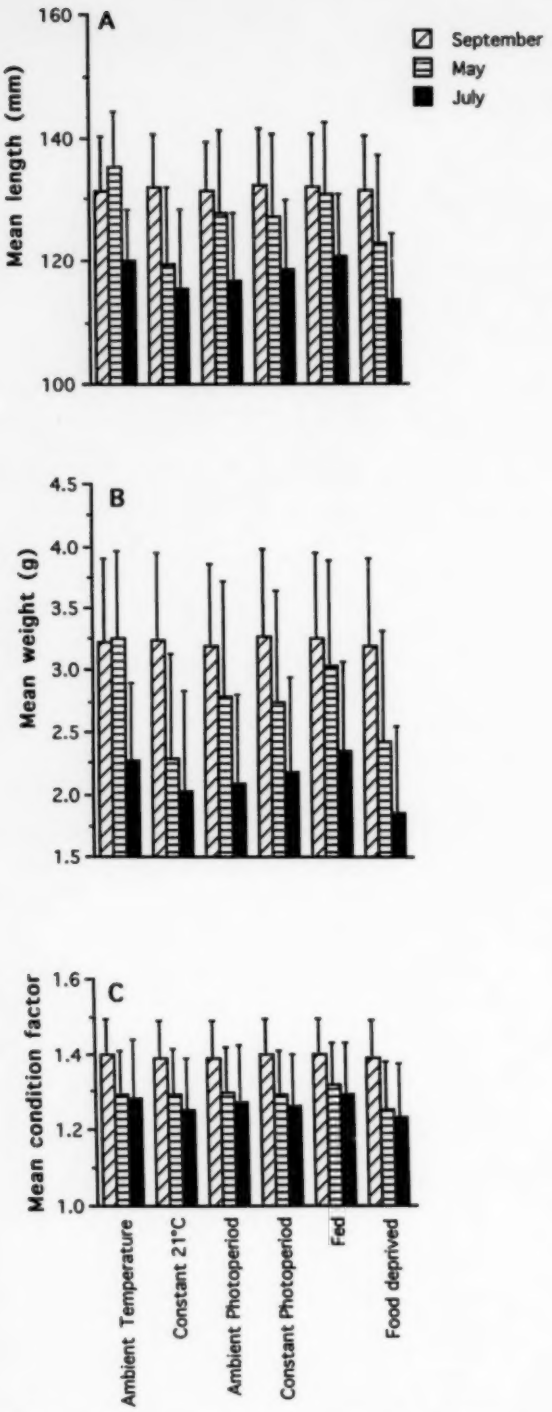

FIG. 2. Changes in the (A) length, (B) weight, and (C) condition factor of larval sea lamprey between September 1991 and July 1992. Data are presented independently for each environmental factor and feeding regime and represent mean values ± 1 SD. Replacement larvae and metamorphosing animals were excluded from the May and July data.

compared with September (Fig. 2). Larvae kept at 21°C or nonfeeding conditions tended to show larger declines in length and weight than animals kept in the ambient temperature or those that were fed. These changes in length and weight appeared to be greater between September and May for larvae deprived of food in the constant temperature compared with larvae held in the ambient regime and fed, for which May–July was the period of greatest change.

Length, Weight, and CF of Metamorphosing Animals

Animals at stages 1–3 of metamorphosis in August 1992 were significantly ($P < 0.05$) larger in size (length and weight) and had a higher CF than unmetamorphosed larvae (Fig. 3). The mean (±SD) length, weight, and CF of stage 2 and 3 metamorphosing animals were 128 (8) mm, 3.2 (0.6) g, and 1.50 (0.11), while unmetamorphosed larvae ($N = 723$) were 116 (11) mm, 2.0 (0.7) g, and 1.23 (0.15). The minimum length, weight, and CF of animals positively identified as metamorphosing (stage 2 or 3) were 111 mm, 1.8 g, and 1.32. These values are lower than was established for field and short-term laboratory animals from the Chippewa population in 1991 (117 mm, 2.8 g, and 1.50; Youson et al. 1993).

We tested two methods for predicting which larvae in September would enter metamorphosis the following July (Table 1). Approximately 11% (48 of 420) of the animals in the ambient temperature regime and none in the constant 21°C temperature entered metamorphosis. Using criteria of 120 mm, 3.0 g, and a CF of 1.50 (Youson et al. 1993), we predicted from the September data that 49 (11.7%) larvae would metamorphose in the ambient temperature and 59 (14%) in the constant temperature. The ratio of metamorphosing to nonmetamorphosing larvae in the ambient temperature is not significantly different ($P > 0.05$) from the expected ratio of 12 (49 of 420) metamorphosing to 88 (371 of 420) nonmetamorphosing larvae for all replicates pooled. However, the number of metamorphosing animals in 3 of the 14 replicate tanks with metamorphosing animals differed significantly ($P > 0.05$) from predictions for these tanks; two tanks had more animals, while the third tank had fewer animals than predicted. The length criterion of 120 mm alone resulted in the largest predictions of metamorphosis, consisting of all animals in each group (420). There were significantly ($P < 0.05$) fewer metamorphosing animals than predicted by this method.

Discussion

The incidence of metamorphosis in larval sea lamprey is influenced by the temperature regime to which premetamorphic larvae are exposed but not by photoperiod or food deprivation. These results are consistent with those from other studies of the influence of water temperature (Purvis 1980; Youson et al. 1993) and photoperiod (Cole and Youson 1981; Youson et al. 1993) on metamorphosis in sea lamprey. Our results using seasonally changing conditions for almost 1 yr imply that the seasonal change in temperature rather than photoperiod is a key environmental factor controlling metamorphosis in appropriately prepared larvae.

Colder temperatures during the winter may be necessary to facilitate final physiological conditioning in immediately premetamorphic sea lamprey, i.e., the deposition of lipid, bringing total body reserves up to about 14% of the wet

body weight by the time the larva enters metamorphosis. Without this conditioning, a larva cannot enter metamorphosis. Much of the lipid deposition in premetamorphic larvae during the arrested growth phase occurs in the fall and winter months prior to metamorphosis (Lowe et al. 1973), indicating that metabolism has shifted from protein synthesis to lipogenesis. In the winter, at high temperature (e.g., 21°C), the metabolic demands were probably of sufficient magnitude to initiate catabolism and reduce lipid stores to levels that were not adequate to support the larva during metamorphosis. This view is supported by the fact that larvae kept at 21°C declined more in length and weight between September and May and did not enter metamorphosis the following summer than larvae in the ambient regime. During spring, when temperature is rising, lipid deposition in premetamorphic larvae is lessened compared with nonmetamorphosing larvae (O'Boyle and Beamish 1977), possibly because feeding by immediately premetamorphic larvae decreases at this time (O'Boyle and Beamish 1977) and basal metabolism increases with temperature (Holmes and Lin 1994), further reducing the storage of assimilated material.

Rising water temperatures in spring appear to be important for the normal initiation of metamorphosis in sea lamprey, since only animals kept in the ambient temperature regime entered metamorphosis. The magnitude of the temperature increase is likely a key determinant of the number of larvae that enter metamorphosis. For example, using larvae from the Big Garlic River, the incidence of metamorphosis increased from 5–10% among animals confined to cages in Lake Superior (7–11°C) to an intermediate rate of 46–76% among animals caged in the Big Garlic River (14–16°C) to 75–100% among animals held in aquariums at 20–21°C (Purvis 1980). The effect of temperature could not be clearly separated from the effects of other environmental factors by Purvis (1980). However, in a laboratory study, Youson et al. (1993) observed that 66% of the animals kept at 13°C and 84% of the animals at 21°C from June to August entered metamorphosis, which is consistent with the intermediate and high temperature ranges used by Purvis (1980).

The ecological significance of the seasonal temperature cycle to holarctic fishes is well known. For example, gonadal maturation in yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum*) occurs during the fall and winter when water temperature is below 10°C and culminates in spawning during periods of rising temperature in spring (Hokanson 1977). Chill durations <185 d from 30 October appear to reduce spawning in these species (Hokanson 1977). Thus, cold water temperatures during the winter for some species-specific period of time allow individuals to prepare physiologically for energetically demanding events such as spawning, while rising temperatures in the spring initiate the event. However, the thermal limits and the length of the exposure period that facilitate physiological conditioning for metamorphosis in lampreys have yet to be determined.

The absence of a long-term photoperiod effect on metamorphosis in sea lamprey is consistent with evidence from a short-term study on the same population (Youson et al. 1993) and earlier evidence from anadromous sea lamprey (Cole and Youson 1981). Laboratory studies on *Lampetra planeri* (Eddy 1969) and anadromous sea lamprey (Cole and Youson 1981), in which the pineal complex was removed, found that metamorphosis was not initiated, indicating that

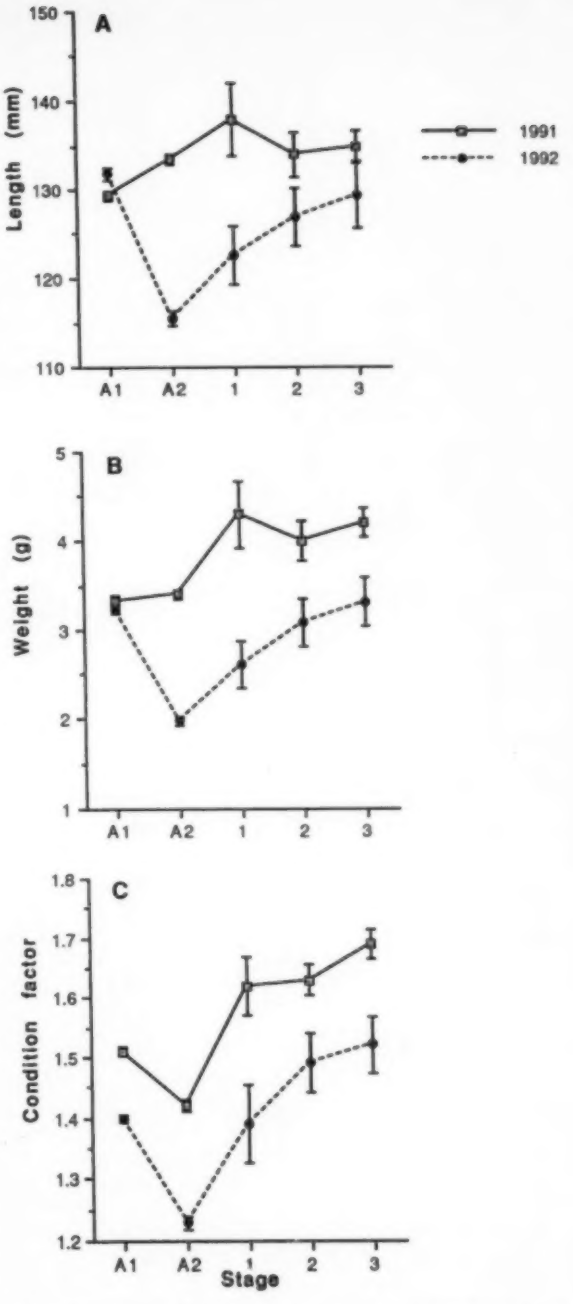

FIG. 3. Comparison of changes in the (A) length, (B) weight, and (C) condition factor of animals at each stage of metamorphosis by August 1991 and 1992. Data represent mean values ± 95% CL. The 1991 data are from a short-term experiment between June and August 1991 (Youson et al. 1993). Stage A1 is larvae at the beginning of each experiment in June 1991 and September 1991, and stage A2 is unmetamorphosed larvae in August 1991 and 1992, respectively. Metamorphosing stages 1–3 are as described in Youson and Potter (1979). Sample sizes in 1991 are as follows: A1, 874; A2, 447; 1, 23; 2, 44; 3, 93. Sample sizes in 1992 are as follows: A1, 841; A2, 723; 1, 21; 2, 25; 3, 22.

TABLE 1. Comparison of the observed and predicted number of metamorphosing sea lamprey from the Chippewa River in two temperature regimes in 1992.

Temperature regime	Total *N*	Predictor	Number metamorphosing	*G* value	*P*
Ambient	420	Observed	48		
		Spring[a]	49	0.02	>0.05
		120 mm[b]	420	1824	<0.05
Constant 21°C	420	Observed	0		
		Spring	59	822	<0.05
		120 mm	420	5074	<0.05

[a]Predictor criteria are 120 mm, 3.0 g, and a CF of 1.50 from Youson et al. (1993).
[b]Presumptive metamorphosing animals had to meet a minimum 120-mm-length criterion only.

the photosensitive pineal complex is an important component regulating the onset of metamorphosis. There is some speculation that the pineal complex may be sensitive to exogenous and endogenous factors other than photoperiod (Youson 1994).

Although the effects of long-term starvation on the morphology and physiology of some organs and tissues resemble changes seen in early metamorphosing animals (Youson 1993), food deprivation had no effect on the number of metamorphosing animals in the present study. However, larvae and metamorphosing animals were significantly (*t*-test, $P < 0.05$) smaller in size and in poorer condition at a given stage of metamorphosis in 1992 compared with animals from the same population in August 1991 (Youson et al. 1993). These differences in size may have implications for the sea lamprey management program in the Great Lakes if survival during metamorphosis and the ability to attack teleosts, particularly the first attack of newly metamorphosed parasites, are dependent on the size of the lamprey.

The absence of metamorphosis among larvae kept at 21°C may be related to a deficiency of lipid stores associated with an increase in metabolic rate and some catabolism, particularly over the winter period, or to our underestimation of the feeding rate. We believe that the former is the most appropriate interpretation of our data, considering the changes in lengths and weights observed over the course of the experiment (Fig. 3). This viewpoint would be strengthened if we had used a true factorial experimental design, but because we probably did not feed larvae an ad libitum diet, we cannot conclusively separate the effects of high temperature and food deprivation in our data. The quantity of yeast fed to the animals ($0.5 g \cdot ind^{-1} \cdot wk^{-1}$) in this and earlier studies (Youson et al. 1993) was based on the results of Hansen et al. (1974). Other long-term laboratory studies (Murdoch et al. 1991, 1992) have fed larval lampreys at rates of 0.6–6.3 $g \cdot ind^{-1} \cdot wk^{-1}$ and reported positive growth in length and weight at approximately the same density (172 larvae$\cdot m^{-2}$) as used in the present study (164 larvae$\cdot m^{-2}$). However, negative growth occurred at a density of 345 larvae$\cdot m^{-2}$, when the larvae were fed 0.63 $g \cdot ind^{-1} \cdot wk^{-1}$ (Murdoch et al. 1992). Mallatt (1983) only observed positive growth in larvae of *L. tridentata* held at densities of 30–150 larvae$\cdot m^{-2}$ when they were fed 8–80 $g \cdot ind^{-1} \cdot wk^{-1}$. The results of another long-term study using the same temperature regimes but a density of 60 larvae$\cdot m^{-2}$ (11 larvae$\cdot$tank^{-1}) fed 1.8 $g \cdot larva^{-1} \cdot wk^{-1}$ were consistent with the present study concerning metamorphosis, but the animals were larger in size and in better condition than the Chippewa River larvae in this study (Holmes and Youson 1994).

Larval sea lamprey in the present study were held at a density of 164 larvae$\cdot m^{-2}$, while density-dependent inhibition of growth has been observed at ≈30 larvae$\cdot m^{-2}$ (Mallatt 1983; Morman 1987; Murdoch et al. 1992). Because the initiation of metamorphosis in lampreys is related to size, density-dependent reductions in growth over the larval period (3–7 yr) would lead to variation in the age of metamorphosing animals, with larvae in low-density habitats metamorphosing at an earlier age than larvae in high-density habitats (Morman 1987). These differences in density have implications for the program to control sea lamprey abundance in the Great Lakes, since the main method, periodic applications of selective toxicants to kill the larvae in their natal streams before they metamorphose, may promote larval growth in reestablished populations and lead to metamorphosis at an earlier age (Purvis 1980).

In this study and Youson et al. (1993), criteria of 120 mm, 3.0 g, and a CF of 1.50 were the best method of identifying premetamorphic larvae likely to metamorphose the following summer. Youson et al. (1993) used these criteria in the spring immediately preceding metamorphosis whereas in the present study, they were applied successfully to data from animals collected in the fall. This finding and the knowledge that the incidence of metamorphosis is greatly influenced by the magnitude of temperature change in the spring (Purvis 1980; Youson et al. 1993) have some practical implications with respect to the control of sea lamprey abundance in the Great Lakes. Stream surveys in the fall are important for the scheduling of lampricide treatments the following year. Scheduling is based on empirical estimates of abundance in the fall prior to treatment and knowledge of when most larvae in the oldest cohort (as a function of the last treatment of a given stream) will metamorphose. The results of Purvis (1980) and Youson et al. (1993) and the present study indicate that metamorphosis will be initiated by more animals when the spring is warm (i.e., the temperature rise is large) than in a cool spring. Thus, the sensitivity of immediately premetamorphic larvae to temperature may account for some of the variation in the timing of metamorphosis and in the number of individuals migrating into the Great Lakes from different streams or from a given stream from year to year.

In summary, water temperature was the only environmental factor influencing the incidence of metamorphosis

in sea lamprey during our study. Cold temperatures during the winter allow physiological conditioning which culminates in the initiation of the metamorphosis during the period of rising temperature the following spring. Keeping larvae at a fixed temperature of 8–10°C rather than 21°C would probably produce the same results with respect to metamorphosis, i.e., no metamorphosis, but these larvae would be larger in size and in better condition than those kept at 21°C. However, in this case the absence of metamorphosis would be more clearly related to the lack of a cue from rising water temperature in the spring period. The finding that photoperiod did not significantly affect metamorphosis is consistent with other studies (Cole and Youson 1981; Youson et al. 1993). Similarly, food deprivation did not have a detectable effect on the incidence of metamorphosis. We suspect that premetamorphic animals had shifted their metabolic strategy from protein synthesis to lipogenesis by the fall in preparation for the nontrophism of metamorphosis and were able to survive food deprivation. We suggest that the size criteria of 120 mm and 3.0 g combined with a CF of 1.50 can be used in fall surveys to identify larvae in a population likely to metamorphose the following summer.

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A Test of Hypotheses to Explain the Sigmoidal Relationship between Total Phosphorus and Chlorophyll *a* Concentrations in Canadian Lakes

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We tested five hypotheses to explain the sigmoidal relationship between total phosphorus (TP) and chlorophyll *a* (Chl *a*), two assuming that the nonlinearity is an artifact of various measurement biases and three assuming that it is based on underlying ecological interactions. Our first hypothesis was rejected; accounting for differences in extraction protocol of Chl *a* among published studies did not affect the sigmoidality. Our second hypothesis could not be rejected; there was an uncoupling between Chl *a* and PHYTO in oligotrophic lakes which may explain the initial nonlinearity. Our third hypothesis was upheld; the initial nonlinearity may be attributed to the presence of a disproportionately large fraction of unavailable phosphorus, since Chl *a* varied linearly with the proportion of total biologically active phosphorus in the TP fraction. The proportion of filamentous cyanophytes varied significantly with TP concentrations, and this was consistent with our fourth hypothesis that the higher Chl *a*:TP ratio at intermediate TP concentrations is attributable to reduced grazing impact of zooplankton in more productive lakes. Finally, Chl *a* varied linearly with total nitrogen, and this was consistent with our fifth hypothesis that the departure from linearity at extremely high phosphorus concentrations is indicative of nitrogen limitation.

Nous avons testé cinq hypothèses pour expliquer la relation sigmoïdale entre le phosphore total (PT) et la chlorophylle *a* (Chl *a*), dont deux supposant que la non-linérarité était un artefact de divers biais des mesures, et trois supposant qu'elle était basée sur des interactions écologiques sous-jacentes. La première hypothèse a été rejetée; le fait de tenir compte des différences des protocoles d'extraction de la Chl *a* entre les diverses études publiées ne modifiait pas la sigmoïdalité. La deuxième hypothèse n'a pu être rejetée; il existe un découplage entre la Chl *a* et PHYTO dans les lacs oligotrophes, qui pourrait expliquer la non-linéarité initiale. Notre troisième hypothèse a été maintenue; la non-linéarité initiale peut être attribuée à la présence d'une fraction proportionnellement importante de phosphore non disponible, étant donné que la teneur en Chl *a* variait de façon linéaire avec la proportion de phosphore total biologiquement actif de la fraction PT. La proportion de cyanophytes filamenteux variait de façon significative avec les concentrations de PT, et ceci était conforme à notre quatrième hypothèse, selon laquelle le rapport plus élevé Chl *a* : PT aux concentrations intermédiaires de PT peut être attribué à un impact réduit du broutage du zooplancton dans les lacs plus productifs. Enfin, la Chl *a* variait de façon linéaire avec l'azote total, et ceci correspondait à notre cinquième hypothèse, selon laquelle l'écart par rapport à la linéarité aux concentrations extrêmement élevées de phosphore peut indiquer une limite pour l'azote.

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In recent years, several groups of investigators have demonstrated that the relationship between algal chlorophyll *a* (Chl *a*) and the primary limiting nutrient in lakes, total phosphorus (TP), is best described by a sigmoidal rather than a linear function (McCauley et al. 1989; Prairie et al. 1989; Chow-Fraser 1991). This nonlinearity suggests that Chl *a* increases more rapidly with nutrients at intermediate concentrations compared with either high or low concentrations. Watson et al. (1992) have also shown that the TP – phytoplankton biomass (PHYTO) relationship is likewise sigmoidal by demonstrating that there is consistency in the Chl:PHYTO ratios across the trophic gradient.

To test the robustness and generality of their models, limnologists in previous studies deliberately used data from published sources that included a wide range of environmental conditions, a large geographical region, and a variety of sampling protocols. Logistical difficulties prevented concurrent measurements of all their variables in all cases and this may have led to invalid comparisons among different statistical models of disparate data subsets. A greater objection

is that analytical procedures for Chl *a* measurements varied from study to study and these procedures may have resulted in differential extraction efficiencies of chlorophyll, and thus contributed to some unknown bias towards evaluation of the nutrient–Chl curve. A brief review of the literature shows that the chemicals used to extract Chl *a* (see Table 1), the extraction duration, as well as the use of either a spectrophotometer or a fluorometer (e.g., SCOR-UNESCO 1966; Holm-Hansen and Riemann 1978; Marker et al. 1980) all have significant effects of Chl *a* estimates. Another problem is that in routine monitoring, only Chl *a* is estimated even though other chlorophylls, especially Chl *b* and Chl *c*, are present in various proportions in natural algal communities. Therefore, estimates of Chl *a* alone may be inadequate where there is variation in species mix and taxonomic composition of the phytoplankton.

A number of hypotheses can be invoked to explain the sigmoidal relationship between TP and Chl *a*, only some of which have a biological or ecological basis. In this paper, we evaluate the applicability of five hypotheses. The first two are based on the assumption that the nonlinearity is actually an artifact resulting from measurement biases and an uncoupling between PHYTO and Chl *a*; the remaining three assume that the nonlinearity is real and are based on underlying ecological mechanisms. By testing these hypotheses, we intend to provide a clearer understanding of the underlying reason(s) for a nonlinear response between nutrient and phytoplankton concentrations in lakes.

Hypotheses to Explain Sigmoidality

(1) Our first hypothesis is that the sigmoidality may simply reflect measurement error associated with the different analytical techniques that have been used to measure Chl *a*. If such is the case, then, calibrating Chl *a* measurements to some standard protocol may eliminate the nonlinearity.

(2) Our second hypothesis is that there is a systematic uncoupling between Chl *a* and PHYTO that depends on a shift in the taxonomic makeup of the algal communities such that the phytoplankton at both ends of the productivity gradient is associated with ratios of Chl *a*:total Chl that are lower than those in the intermediate range.

(3) Our third hypothesis is that the initial nonlinearity in the curve is attributed to the presence of a disproportionately large fraction of phosphorus that is not available for algal growth. If this is true, then plotting Chl *a* against total biologically active phosphorus (TBAP) (as indicated by laboratory algal assay) rather than TP may produce a linear response.

(4) Our fourth hypothesis is that the lower Chl *a* concentrations associated with oligotrophic lakes is attributed to disproportionately high grazing impact of the herbivore community, whose "top-down" (after McQueen et al. 1986) effects decrease systematically as lakes in productivity due to the corresponding increase in the proportion of interfering filamentous algae (e.g., Chow-Fraser and Sprules 1986).

(5) Our fifth hypothesis is the departure from linearity at extremely high phosphorus concentrations is indicative of nitrogen limitation. This expectation is based on observations that in lakes with extremely high TP concentrations, total nitrogen (TN):TP ratios tend to be low (<14; Downing and McCauley 1992), and in these lakes, nitrogen limitation appears to be the rule.

TABLE 1. Summary of the interactive effects of extractant used and type of algae in samples. Numbers refer to studies in which the chemicals named were more efficient than 90% acetone for extracting Chl *a* from samples dominated by the respective algal groups. Methanol refers to 100% methanol as well as chloroform–methanol. 1, Shoaf and Lium (1976); 2, Holm-Hansen and Riemann (1978); 3, Stauffer et al. (1979); 4, Burnison (1980); 5, Marker et al. (1980); 6, Nusch (1980); 7, Wood (1985); 8, Speziale et al. (1985).

	Chemical extractant			
Dominant group	DMSO	DMF	EtOH	MeOH
Blue-greens	3, 8	3, 8	5	2, 3, 7
Greens	1, 3, 4, 8	8	5, 6	6, 7
Chrysophytes				7
Diatoms	3			2, 7

Methods to Test Hypotheses

Hypothesis 1

To test the first hypothesis, we assembled a database from published and unpublished sources that had contemporaneous measurements of TP and Chl *a* (Table 2). To ensure that the database would be sufficiently homogeneous to permit a valid comparison, we used only lakes that were sampled at least 5 times during the ice-free season (generally between May and October, except for coastal lakes in British Columbia where the ice-free season sometimes extended from March to November; Stockner et al. 1980; Stockner and Shortreed 1985). Hanna and Peters (1991) found that in cross-sectional studies, the strongest within-lake differences could be attributed to sampling date rather than to sampling method or sampling sites within a lake, and this indicated that comparisons should only be made for lakes that are sampled with roughly the same sampling frequency and intervals. Since we were interested in testing the variable effects of different extraction procedures on Chl *a* measurements, we included only those studies for which extraction techniques have been adequately documented to permit calibration. Finally, we only included north-temperate lakes to avoid any confounding effects of large climatic differences. Using these criteria, we were able to assemble data for 119 lakes (152 lake-years) from published sources as well as several unpublished sources within Canadian provincial and federal agencies.

Not all of the waterbodies included in this database can be described as "typical" lakes, although they are all lake-like. A notable example is Cootes Paradise Marsh (Table 2; "Y" in Fig. 1), which is a large degraded urban wetland located in Hamilton, Ontario. In its present state, it acts very much like a shallow hypereutrophic lake, with less than 10% cover of emergent or submergent aquatic vegetation. Inclusion of this marsh in the present analysis broadened our range of trophic states and offered us a heuristic if not predictive model of how Chl *a* behaves at extremely high TP concentrations.

In order to calibrate existing measurements to that of a standardized protocol, we conducted an investigation to determine the extent to which the different methods from documented studies affect Chl *a* measurements. Specifically, we wanted to compare the extraction efficiency of

TABLE 2. Description of study lakes included to test the sigmoidal shape of the TP–Chl *a* curve. See text for explanation of abbreviations for protocols. Chl *a* values are in μg/L.

Location	Sources	Number of lakes	Extraction protocol	Correction for phaeophytin	Range of Chl values	Years sampled
Alberta	Chow-Fraser and Trew, 1990; Naquadat files of Alberta Environment (D. Trew, unpublished data)	57	ACET-96-F	Yes	1.1–86.6	1981–88
	Atlas of Alberta lakes (Mitchell and Prepas 1990)	5	EtOH-S	No	1.7–10.2	1981–86
British Columbia	Stockner et al. 1980; Shortreed and Stockner 1981; Stockner and Shortreed 1985	24	ACET-24-F	No	0.6–3.3	1978–80
Experimental Lakes Area, northwestern Ontario	Freshwater Institute (D. Findlay, B. Hecky, M. Stainton, unpublished data)	8	ACET-24-F ACET-24-S	No	0.8–15.2	1974–83
South-central Ontario	Zimmerman et al. 1983	24	ACET-24-S	Yes	0.8–7.5	1980
Cootes Paradise Marsh (western end of L. Ontario)	Painter et al. 1991	1[a]	ACET-24-S	No	22.2–684	1973–90

[a]Two stations were sampled in Cootes Paradise.

different chemicals for the same sample, as well as compare the relative efficiency of a given chemical for samples with different taxonomic compositions from different lakes. The chemicals we chose were intended to represent many of the commonly used solvents besides 90% acetone that are known to give higher yields of Chl *a* for samples dominated by filamentous green and blue-green algae (see Table 1). These included *N*,*N*-dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), and methanol. We did not include ethanol in our comparison because Marker et al. (1980) indicated that of the two alcohols, methanol was used more widely. Additionally, Prepas and Trew (1983) found no significant differences in performance when ethanol was compared with 90% acetone. We also compared the performance of the spectrophotometer versus the fluorometer in calculating Chl *a* values for the same samples to quantify differences between photometric and fluorometric data.

For this comparison, three waterbodies from the large database were sampled to obtain a range of Chl values from 1 to 90 μg/L. These included an oligotrophic reservoir (Gleniffer Reservoir (GL); TP = 9 μg/L), the south and north basins of a eutrophic lake (Baptiste South (BS) and Baptiste North (BN); TP = 55 and 65 μg/L, respectively), and a hypereutrophic shallow lake (Dried Meat (DM); TP = 450 μg/L). To avoid confounding effects of season, we visited all the lakes over a 48-h period in July 1992. Logistical considerations prevented us from sampling a larger number of lakes. Triplicate samples were collected in amber polyethylene bottles and kept cool until filtration (within 12 h of sampling). Appropriate aliquots of composite samples from each lake (200–1000 mL) were filtered through Gelman GF/C, wrapped in aluminum foil, and kept frozen until different extraction protocols were carried out. We used four suites of chemicals to extract the Chl lawns: 90% alkaline acetone, 100% methanol, DMSO with acetone, and DMF with acetone.

Filters were placed in 10 mL of 90% acetone or 100% methanol and extracted for 24 h at below 0°C (ACET-24 and MeOH), while one set was placed in 90% acetone and extracted for 96 h at below 0°C (ACET-96). These samples were subsequently homogenized and centrifuged at 4000 rpm for 5 min. The solvent was decanted into a cuvette and analyzed with a spectrophotometer (Milton Roy 1201). Absorbances were read at 665 and 750 nm. Each extract was then acidified with two drops of 0.3 N HCl and allowed to mix for 3 min. Absorbance was read at 750 and 665 nm after acidification. We used Eq. 1 from Speziale et al. (1984) to calculate Chl *a* concentrations in the samples. All samples were subsequently analyzed fluorometrically with a Turner model 111 fluorometer according to the procedures of Yentsch and Menzel (1963) as modified by Holm-Hansen et al. (1965). To facilitate comparison, both corrected and uncorrected Chl *a* values were calculated in this study.

Methods used to measure TP, PHYTO, and Chl *a* in this large database are documented elsewhere as noted in Table 2. Since we are only concerned here with calibrating Chl *a* measurements, we outline below the four different protocols that have been used to obtain Chl *a* values in the large database: (1) extraction with acetone for 96 h followed by fluorometric analysis (ACET-96-F), (2) extraction with acetone for 24 h followed by fluorometric analysis (ACET-24-F), (3) extraction with ethanol followed by spectrophotometric analysis (EtoH-S, and (4) extraction with acetone for 24 h followed by spectrophotometric analysis (ACET-24-S).

Hypothesis 2

To test Hypothesis 2, we required contemporaneously measured PHYTO data in addition to the Chl *a* and TP values. This reduced our dataset to only 89 lake-years. Since another component of this hypothesis is that the taxonomic makeup of phytoplankton varies predictably with lake trophic status, we also required information on the species composition of algal communities. This procedure further reduced our working database to 33 lake-years. Raw counts and cell dimensions corresponding to each sampling date (at least five times over the sampling season) for each of the 33 lakes

were obtained from Alberta Environment (D. Trew) and the Freshwater Institute (D. Findlay). Biomass values were subsequently calculated and sorted into seven major taxonomic groups: cyanophytes, chlorophytes, euglenophytes, chrysophytes, diatoms, cryptophytes, and dinoflagellates (see Appendix).

Hypothesis 3

To test the third hypothesis, we used concurrent measurements of TP, TBAP, and Chl *a* concentrations from 31 of the study lakes in Alberta. To calculate TBAP, a relationship between orthophosphate and algal biomass was derived in the laboratory and was subsequently used to estimate the amount of phosphorus utilized from test water by a given stock culture of *Selenastrum capricornutum* (supplied by USEPA, Oregon). The relationship was developed which included a range of phosphorus concentrations between 3 and 186 μg/L ($r = 0.98$; $P < 0.01$). Algal biomass was measured by Coulter Counter (model ZBI) following a preliminary calibration with direct microscopic counts and a fluorometer.

The algal assay involved autoclaving flasks of the test lake water for 20 min at 15 psi (1 psi = 6.895 kPa) and 121°C. All flasks were weighed before and after autoclaving and the appropriate quantity of sterile double-distilled water was added to compensate for differences created by evaporation. One millilitre of stock *Selenastrum* was added to 29 mL of autoclaved test water and this was allowed to grow for 96 h at 24°C in continuous illumination (2100 lx). The *Selenastrum* inoculum was prepared from actively growing stock cultures (1–2 wk old). A subsample was dispensed into a sterile tube and centrifuged at 1000*g* for 10 min. The supernatant was subsequently replaced by sterile double-distilled water and the final density was adjusted to 1.8×10^5 cells/mL with a fluorometer. Growth of the test cultures was monitored using in vivo chlorophyll fluorescence until the stationary growth phase was identified (at least 96 h after inoculation). At that time, 0.5 mL of the sample was preserved with Lugol's iodine and later enumerated with the Coulter particle counter.

Hypothesis 4

As an alternative, investigators in the past have assigned algae to functional rather than taxonomic groups (e.g., Sprules and Knoechel 1984) to study the ecological relationships between adjacent trophic levels. However, the majority of these studies have focused on separating algae into two main groups: "edible" and "inedible" based only on a size criterion (e.g., Chow-Fraser and Knoechel 1985; Carpenter et al. 1991; Watson et al. 1992). This approach ignores the fact that other factors such as algal shape, taste, and colony morphology can also affect the edibility of algae (DeMott 1986; Knisely and Geller 1986; Vanderploeg 1990; Chow-Fraser and Maly 1992) and that the "inedible" category is an umbrella group that contains large noningestable phytoplankton as well as filamentous algae, which in addition to being inedible are also known to interfere with the grazing activities of zooplankton (Chow-Fraser and Sprules 1986; Hawkins and Lampert 1989). In this study, algae have been grouped according to size, shape, and colonial morphology as follows: (1) cells <10 μm, (2) cells or colonies 10–30 μm, (3) cells >30 μm, (4) colonies >30 μm, and (5) filamentous algae (see Appendix).

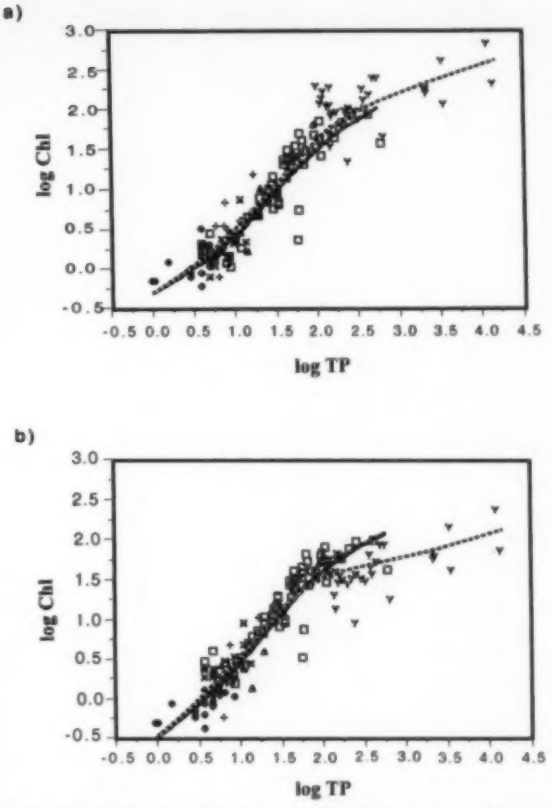

FIG. 1. Plot of $\log_{10}$ Chl (μg/L) versus $\log_{10}$ TP (μg/L) for (a) unstandardized data and (b) standardized data. Lines were obtained by applying a smoothing spline procedure to the data (SAS JMP, SAS Institute, North Carolina; lambda = 1). Data are for 119 waterbodies and include 152 lake-years. Symbols: X, from Zimmerman et al. (1983); Y, from Painter et al. (1991); +, from Freshwater Institute (Experimental Lakes Area); □, from Alberta Environment; △, from Mitchell and Prepas (1990); ◇, from Stockner et al. (1980) and Stockner and Shortreed (1985); broken line, all data; solid line, relationship excluding Cootes Paradise Marsh (Y).

Hypothesis 5

In most cases, contemporaneous measurements of TN were available for lakes in the large database and these were used to test the last hypothesis that TN rather than TP is a better predictor of Chl *a* across the entire range of trophic conditions.

In this paper, data were appropriately log-transformed or arcsin-transformed before they were subjected to statistical analyses. The base for all log-transformations was 10. For convenience, the base will be omitted in the remainder of the text.

Hypotheses and Tests

To test the first hypothesis, we first used a smoothing spline procedure (SAS JMP, SAS Institute, North Carolina; lambda = 1) to determine the shape of the best-fit curve through the log-normalized TP-Chl *a* data of the large database

TABLE 3. Summary of mean Chl *a* values (μg/L; $n = 3$), corrected for phaeopigments. Numbers in parentheses are 1 SE of the mean. See text for explanation of abbreviations.

Lake	Machine	ACET-24	ACET-96	DMF-A	DMSO-A	MeOH
GL	F	0.96	1.35	2.19	1.68	1.05
		(0.106)	(0.078)	(0.538)	(0.171)	(0.253)
	S	1.54	2.05	1.78	1.86	2.25
		(0.365)	(0.295)	(0.289)	(0.399)	(0.685)
BS	F	49.69	57.82	58.90	60.89	49.46
		(3.812)	(1.506)	(0.163)	(3.122)	(5.273)
	S	60.65	63.06	70.41	83.13	91.12
		(2.571)	(1.676)	(0.750)	(1.464)	(7.637)
BN	F	63.15	67.98	70.63	66.26	53.95
		(0.732)	(0.614)	(4.125)	(1.464)	(8.888)
	S	81.65	73.66	86.98	81.21	103.54
		(3.766)	(1.202)	(5.831)	(1.569)	(12.739)
DM	F	83.63	90.91	84.66	84.89	73.85
		(3.807)	(2.438)	(0.162)	(7.840)	(0.845)
	S	122.04[a]	103.94	110.05	109.16	136.08
			(1.506)	(0.754)	(11.055)	(2.719)

[a]No SE calculated because $n = 2$.

($n = 119$ lakes; 152 lake-years). This produced a sigmoidal relationship which accounted for almost 92% of the variation in the data (broken line, Fig. 1a; $r^2 = 0.92$). By comparison, a linear regression analysis performed on the same dataset yielded a relationship that only described 87% ($r^2 = 0.87$; $P < 0.0001$) of the variation. These results, which are consistent with those of McCauley et al. (1989) and Prairie et al. (1989), confirm that the relationship between reported TP and Chl *a* is best described by a sigmoidal rather than a linear equation.

Our first hypothesis concerns the differential effects of various analytical procedures on the extraction efficiency of Chl *a* in samples collected from lakes with a wide range of trophic states. To address this, we conducted a three-factor experiment to determine the effects of instrument type (spectrophotometer versus fluorometer), chemical extractant/extraction duration (ACET-24, ACET-96, DMSO-A, DMF-A, and MeOH; see Methods to Test Hypotheses), and lake origin on Chl *a* values (Table 3). A nested analysis of variance indicated that each variable explained a significant amount of variation in the data. Lake origin alone, however, explained close to 81% of the variation ($P < 0.0001$). Inclusion of the nested effect of instrument type in the model explained 6% of the variation ($P < 0.0001$), while the effect of extraction protocol explained an additional 6% ($P = 0.0015$).

When the effects of lake origin and chemical extractants were factored out, photometric data were consistently higher than fluorometric data except for one treatment (GL samples extracted with DMF-A; Table 3). The magnitude of this disparity ranged from 47% (GL-MeOH treatment) to 92% (BS-ACET-96) with majority of the discrepancies between 75 and 85%. Schanz and Rai (1988) also reported similar discrepancies between fluorometric and photometric data and attributed these differences to interference from carotenoids which produced overestimates when a spectrophotometer was used and underestimates when a fluorometer was used. Other investigators had also noted that Chl *b* causes overestimation of phaeopigments by fluorometry, thus producing lower acid ratios and consequently lower Chl *a* values (e.g., Yentsch 1965; Stauffer et al. 1979).

There was least agreement between fluorometric and photometric data when MeOH was used, probably because the acidification step causes the absorption maximum of phaeophytin *a* a shift from 665 nm in MeOH and thereby leads to underestimates of phaeopigments on the one hand and overestimates of Chl *a* on the other (Nusch 1980). The problem was not apparent with the other extraction mixtures because phaeophytin is more stable in acetone. Thus, the almost twofold higher photometric measurements corresponding to MeOH extraction are largely because we excluded a neutralization step in our protocol (Holm-Hansen and Riemann 1978). Another reason may be because Chl *b* fluoresces close to the same wavelength as Chl *a* in MeOH but not in acetone (Nusch 1980), and this may also have inadvertently led to comparatively low Chl values in the methanolic extracts when the fluorometer was used.

Fluorometric and photometric techniques also differed with respect to the ratio of uncorrected to corrected Chl *a* values (UNCORR:CORR; Fig. 2a and 2b, respectively). Regardless of instrument type used, the ratios corresponding to the MeOH treatment tended to be low, probably because of the problem with acidification as discussed previously. With the exception of the methanolic extracts, uncorrected Chl *a* concentrations were consistently twice those of corrected values when samples were analyzed with the fluorometer. By comparison, samples analyzed with the spectrophotometer were a great deal more variable, with ratios ranging from less than 2 to greater than 4.

Cross-study comparisons using different measurement protocols are inappropriate because differences in analytical techniques produced significantly different Chl *a* values in parallel samples (Table 3). Studies also differed in the treatment of phaeophytin interference, and since only some of the investigators in Table 2 reported corrected Chl *a* values, this may have contributed another source of error to the cross-study comparison. Therefore, we sought to calibrate the published data to a common unit by standardizing all reported measurements to phaeophytin-corrected Chl values, equivalent to those that had been obtained photometrically following DMSO-A extraction (Table 4). We chose this because

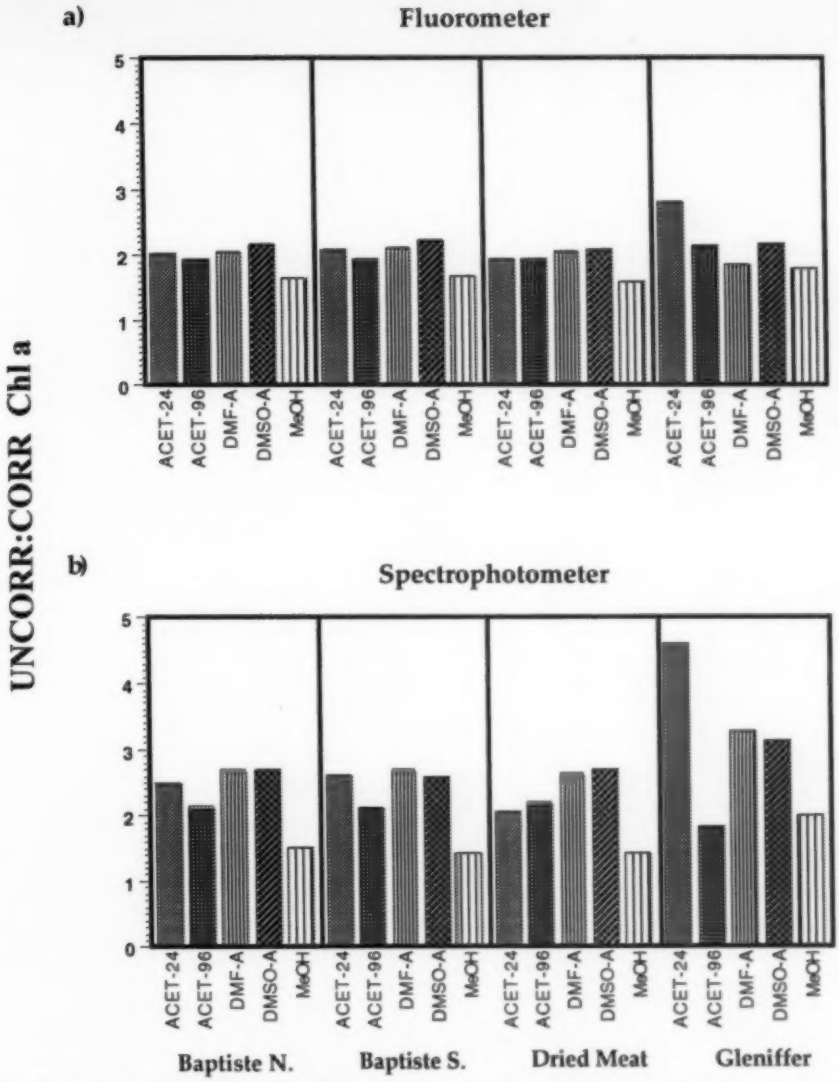

FIG. 2. Ratio of uncorrected (UNCORR; for phaeophytin) to corrected (CORR) Chl *a* values for different extraction protocols that had been measured with (a) a fluorometer and (b) a spectrophotometer. See Table 1 for explanation of abbreviations for protocols.

we had not included the EtOH-S method (used by Mitchell and Prepas 1990) in our earlier comparison of extraction protocols, but Webb et al. (1992) performed a direct comparison of data obtained with the EtOH-S method and a photometric protocol involving DMSO-A extractions. We used information from Webb et al.'s (1992) study to calculate a conversion factor of 0.78 that relates uncorrected data obtained by the EtOH-S method to corrected Chl concentration obtained photometrically by a method that is similar to our DMSO-A method.

We then used information from our own experiments (Table 3; Fig. 2) to derive the remainder of the conversion factors to standardize the other published Chl values (Table 4). In deriving these conversion factors, we considered both the extraction protocol as well as the trophic status of the lakes in question. This meant that we used experimental data (Table 3) corresponding to GL, BN and BS, and DM, respectively, to calculate conversion factors for lakes with low (TP < 10 μg/L), medium (TP between 10 and 100 μg/L), and high (TP > 100 μg/L) productivity. For example, in the low productivity category, we used data corresponding to GL samples and determined that photometric data extracted with DMSO-A were on average 1.38 times higher than corresponding fluorometric data that had been extracted in acetone for 96 h (corrected for phaeophytin).

The standardized Chl *a* values were then plotted against log TP. The same smoothing spline procedure that had yielded an obviously sigmoidal curve for the unstandardized data (broken line, Fig. 1a) produced a line with a reduced curvature in the initial portion of the curve and a more flattened line

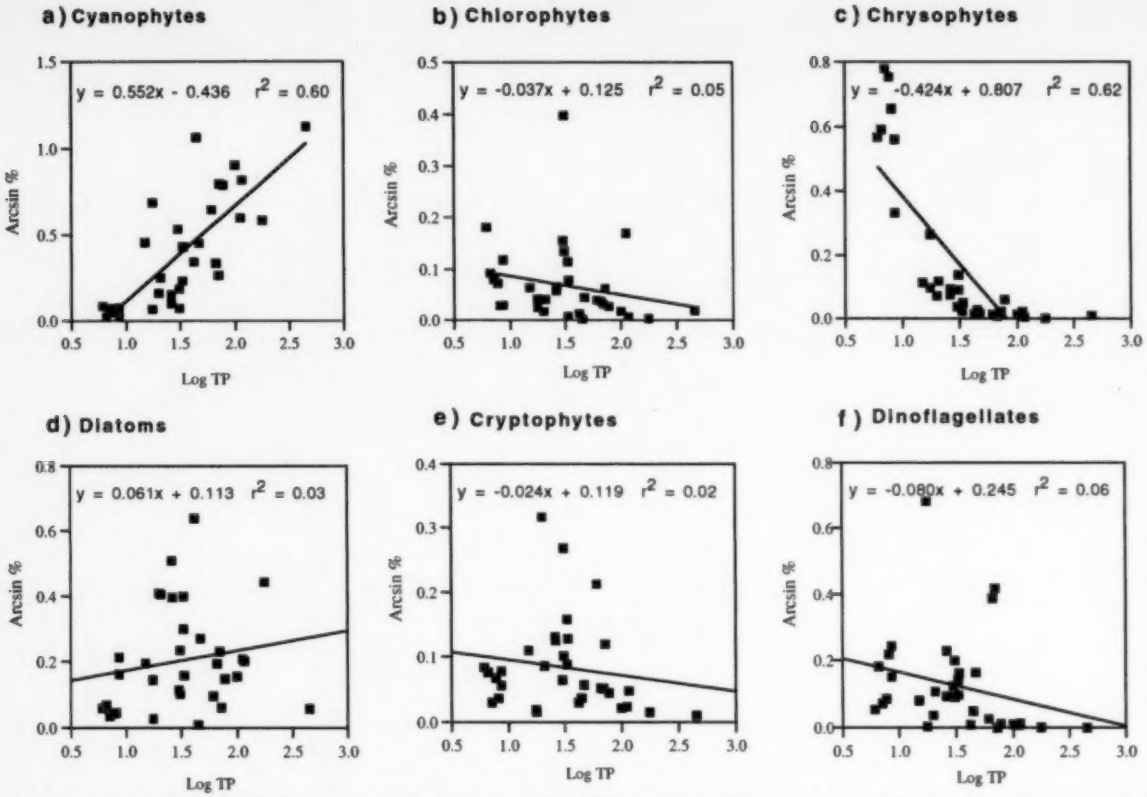
a) Cyanophytes
y = 0.552x - 0.436 r² = 0.60
b) Chlorophytes
y = -0.037x + 0.125 r² = 0.05
c) Chrysophytes
y = -0.424x + 0.807 r² = 0.62
d) Diatoms
y = 0.061x + 0.113 r² = 0.03
e) Cryptophytes
y = -0.024x + 0.119 r² = 0.02
f) Dinoflagellates
y = -0.080x + 0.245 r² = 0.06
Arcsin %
Log TP

FIG. 3. Proportion of total phytoplankton biomass (arcsin-transformed) plotted as a function of $\log_{10}$ TP (μg/L) for (a) cyanophytes, (b) chlorophytes, (c) chrysophytes, (d) diatoms, (e) cryptophytes, and (f) dinoflagellates. Data are seasonal mean values calculated for 33 lakes in Ontario (Experimental Lakes Area) and Alberta.

TABLE 4. Correction factors used to calculate a standardized Chl value for valid comparisons. Productivity categories were designated as follows: low, lakes with Chl < 10 μg/L; medium, Chl between 10 and 60 μg/L; high, Chl >60 μg/L. See text for explanation of how correction factors were calculated. n/a, not applicable.

Source	Factors used for each category		
	Low	Medium	High
Alberta Environment (D. Trew, unpublished data)	1.381	1.318	1.125
Atlas of Alberta Lakes (Mitchell and Prepas 1990)	0.780	n/a	n/a
Stockner et al. 1980; Shortreed and Stockner 1981; Stockner and Shortreed 1985	0.693	n/a	n/a
Freshwater Institute (M. Stainton, unpublished data)	0.693	n/a	n/a
Zimmerman et al. 1983	1.212	1.155	n/a
Painter et al. 1991	n/a	0.399	0.348

in the latter portion for the standardized data (broken line, Fig. 1b). Overall, the standardized relationship explained only marginally less variation (r^2 = 0.90) compared with the unstandardized data (r^2 = 0.92), but consistent with earlier analyses, a least-squares linear regression procedure resulted in a statistically poorer fit (r^2 = 0.79). Thus, accounting for errors due to differential extraction efficiencies, different analytical technique, and different treatment of phaeophytin content in samples did not appear to alter the nonlinear relationship between TP and Chl *a*.

Since all of the extremely high values of TP (>300 μg/L) and Chl (>100 μg/L) were associated with only one source, and this source was not a typical lake (Cootes Paradise Marsh; denoted by "Y"; see discussion in Methods to Test Hypotheses), we excluded the marsh data and fit the curve through the remainder of the data to determine the extent to which our original results had been biased. In the case of unstandardized data, the curve through the reduced dataset was essentially the same (solid line, Fig. 1a), although the overall truncated presentation does not give as strong an impression of sigmoidality as does the curve through the entire dataset (broken line, Fig. 1a). In the case of standardized data, however, the curve through the reduced dataset (solid line, Fig. 1b) did not level off at the same point as did the curve through the larger dataset (broken line, Fig. 1b), suggesting that application of the conversion factors to calibrate data for Cootes Paradise Marsh may have been inappropriate. Nevertheless, the strong sigmoidal shape of the TP–Chl *a* curve was retained whether or not we included data from the marsh.

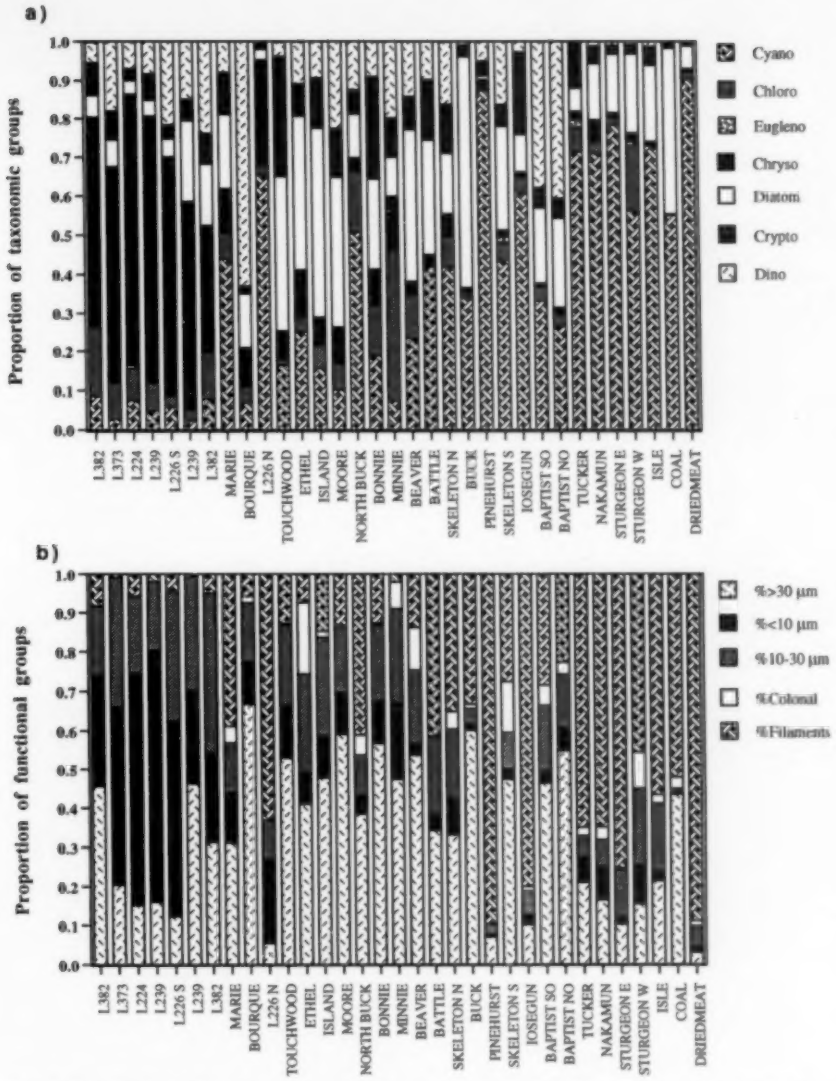

FIG. 4. Distribution of (a) seven taxonomic groups and (b) five functional groups for 33 lakes in Ontario (Experimental Lakes Area) and Alberta. Data are means of at least five sampling trips during the ice-free season. Lakes are sorted by ascending TP concentrations.

In addressing our second hypothesis, we first tested the assumption that the taxonomic makeup of phytoplankton changed predictably across the trophic gradient. Owing to availability of limited data, algal biomass from only 33 of the 199 lakes could be used to test this hypothesis (raw data in Appendix). The seasonal mean percent distributions of six major taxonomic groups in each of the 33 lakes were arcsin-transformed and plotted against log TP (Fig. 3). The proportion of blue-greens increased significantly with TP concentration, while those of chrysophytes decreased significantly ($r^2 = 0.60$ and 0.62, respectively; $P < 0.05$). There was no apparent pattern in the distribution of the remaining taxonomic groups although diatoms and cryptophytes seemed to have been more abundant at intermediate TP concentrations.

The distribution pattern that emerges from this analysis is a shift in dominance of chrysophytes in oligotrophic communities to one of diatoms, green algae, and cryptophytes in mesotrophic communities to a largely blue-green community in eutrophic and hypereutrophic lakes. This gradient of change in phytoplankton community structure with increasing lake trophic status is better visualized when all of the taxonomic groups are presented together in stacked histograms (Fig. 4a). Canfield et al. (1989) and Duarte et al. (1992) have reported a similar shift in algal community structure for a large number of shallow Florida lakes. They noted that there was a gradual shift from dominance by green algae in oligotrophic lakes to dominance by blue-green algae in eutrophic and hypereutrophic lakes, with a peak in diatom abundance in mesotrophic lakes. The main difference between results of our study and theirs is that chrysophytes were

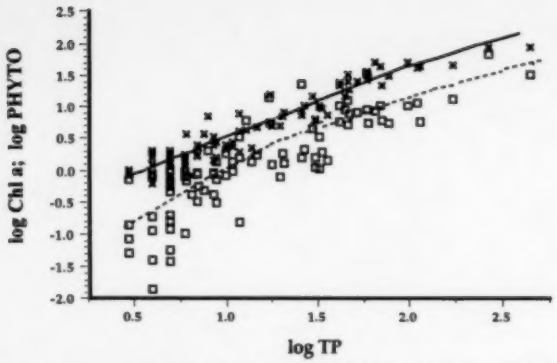

FIG. 5. Log_{10} standardized Chl *a* (X; μg/L) and log_{10} PHYTO (□, mg/L) plotted as a function of log_{10} TP (μg/L). Lines were obtained by applying a smoothing spline procedure to the data (see Fig. 1 legend).

more prominent in unproductive lakes in Canada whereas green algae were more prominent in the corresponding lakes in Florida. That there is such striking similarity in the overall pattern of algal community in these lake sets is remarkable considering the geographical and climatic disparities between the two.

To determine if Chl *a* and PHYTO uncouple along this trophic gradient, we regressed contemporaneous measurements of Chl *a* and PHYTO against TP (n = 89 lake-years; Fig. 5). A spline-fit resulted in a curve through the TP–Chl *a* data that appeared linear, with a corresponding r^2 value of 0.93, which was virtually identical to that of a linear regression analysis (0.92). Similar regression analyses performed on the corresponding TP–PHYTO data also produced more or less linear relations (r^2 = 0.71 and 0.69, respectively) although the residual variation about the lines was much larger. The disappearance of the previously noted sigmoidal TP–Chl *a* curve was obviously the result of truncating the range of TP values (reduced from 152 to 89 lake-years) when we restricted our analysis to only lakes with parallel measurements of Chl and PHYTO. Therefore, merely comparing the shape of the TP–Chl *a* and TP–PHYTO curves was not sufficient to test the second hypothesis.

Although the slopes of the TP–Chl (1.23 ± 0.040) and TP–PHYTO (1.22 ± 0.087) linear regression equations were statistically similar (ANCOVA; $P < 0.05$), there was an obvious uncoupling of Chl and PHYTO at low TP concentrations that is more clearly demonstrated by a sharp decrease in the Chl:PHYTO ratio with TP concentrations above 10 μg/L (Fig. 6a). In contrast with the findings of Watson et al. (1992), we found inconsistencies in the relationship between log Chl *a* and log PHYTO (Fig. 6b; breakpoint at approximately 1 mg/L of phytoplankton biomass). Therefore, although the overall trends in the TP–Chl *a* and TP–PHYTO regressions are similar, there is an uncoupling between Chl *a* and PHYTO at low TP concentrations that may explain why there is a departure from nonlinearity in the initial portion of the TP–Chl *a* curve.

One hypothesis for the uncoupling between PHYTO and Chl *a* is that the ratio of Chl *a* to total Chl increases as lakes become more productive. This may be because cyanophytes, which contain only Chl *a* (as opposed to Chl *b* or *c*), dominate in productive lakes, while chlorophytes and chrysophytes,

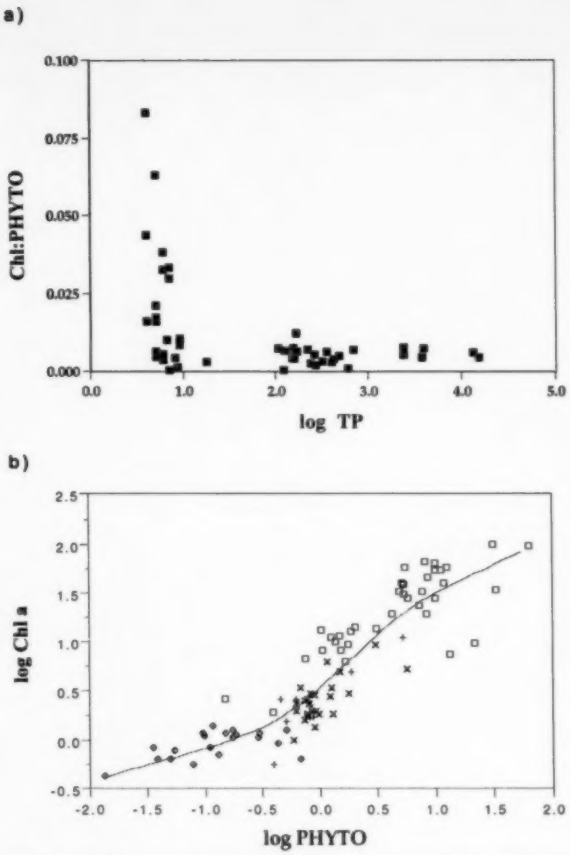

FIG. 6. (a) Ratio of standardized Chl *a* to PHYTO plotted as a function of log_{10} TP (μg/L). (b) Log_{10} standardized Chl *a* (μg/L) plotted as a function of log_{10} PHYTO biovolume (mg/L); the line was obtained by applying a smoothing spline procedure to the data (see Fig. 1 legend).

which usually contain quantities of Chl *b* and *c* (which are known to interfere with the measurement of Chl *a* by producing overestimates of phaeopigments), dominate in unproductive lakes. These two phenomena in concert would likely result in a nonlinear increase in Chl *a* with TP concentrations, even if there is a linear increase in algal biomass within the same range. Future studies should therefore be carried out to determine the relative distributions of the various chlorophyll pigments as lakes increase in trophic status.

To test our third hypothesis, we investigated whether or not the proportion of TBAP in the TP pool changed as lakes increased in productivity. To eliminate any confounding effects due to differences in analytical and measurement protocol, we selected a dataset in which samples had been collected over a 3-wk period during July 1983 and where all Chl *a* had been measured with the ACET-96-F method (31 lakes). The proportion of biologically active phosphorus in the TP pool clearly increased along the trophic gradient (Fig. 7a). The percent TBAP increased from a low of 5% at TP concentrations <10 μg/L to over 85% at TP concentrations in excess of 100 μg/L. At concentrations above 200 μg/L, the proportion of TBAP seemed to decline, but this should be verified with a larger number of hypereutrophic lakes.

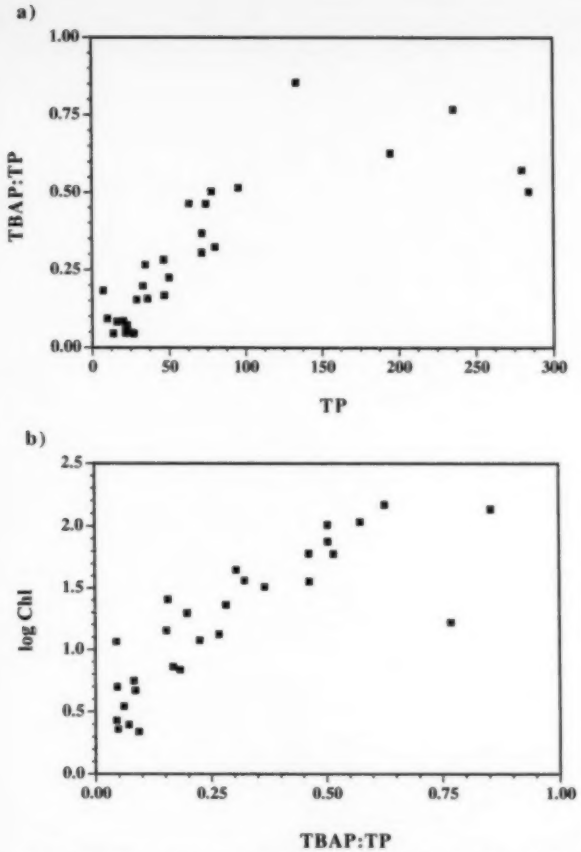

FIG. 7. (a) Plot of the ratio TBAP:TP versus TP (μg/L). (b) Plot of $\log_{10}$ Chl (μg/L) versus the ratio of TBAP:TP for 31 lakes in Alberta.

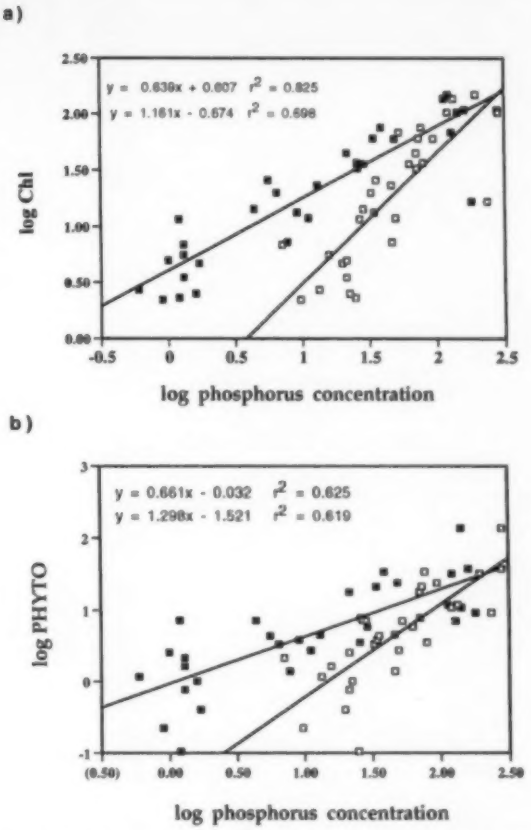
a)
y = 0.639x + 0.807 r² = 0.825
y = 1.161x - 0.674 r² = 0.698
log Chl
log phosphorus concentration
b)
y = 0.661x - 0.032 r² = 0.625
y = 1.298x - 1.521 r² = 0.619
log PHYTO
log phosphorus concentration

FIG. 8 Plot of (a) $\log_{10}$ Chl *a* (μg/L) and (b) $\log_{10}$ PHYTO (mg/L) versus $\log_{10}$ TP (□; μg/L) and $\log_{10}$ TBAP (■; μg/L) concentrations for 31 lakes in Alberta. Lines through the data are the least-squares linear regression equations (all $P < 0.001$).

Corresponding Chl *a* values varied linearly with the proportion of TBAP up to 0.70, and thereafter seemed to plateau (Fig. 7b).

Not surprisingly, there was no obvious indication of sigmoidality in either the TP–Chl or TBAP–Chl relationships (Fig. 8a), since there was only a limited range of phosphorus concentrations. It is worth noting, however, that although TBAP was a much better predictor of Chl *a* than TP (r^2 = 0.83 versus 0.70, respectively), there was no such improvement in the TBAP–PHYTO relationship when compared with that of TP–PHYTO (r^2 = 0.63 versus 0.62, respectively; Fig. 7b). Consistent with earlier analyses (Fig. 6b), a plot of Chl *a* against PHYTO clearly showed a departure from linearity at low phytoplankton concentrations (Fig. 9). A comparison of results from the spline-fit procedure (solid line) and a linear regression analysis (broken line) indicated that the data were better described by a curvilinear than a linear fit (r^2 = 0.85 versus 0.79, respectively). These observations thus provide further evidence that there is an uncoupling between Chl *a* and PHYTO in unproductive systems, and we caution against using them interchangeably throughout the entire productivity gradient without additional study.

To test our fourth hypothesis, algae corresponding to the smaller dataset of 33 lakes (Appendix) were sorted according

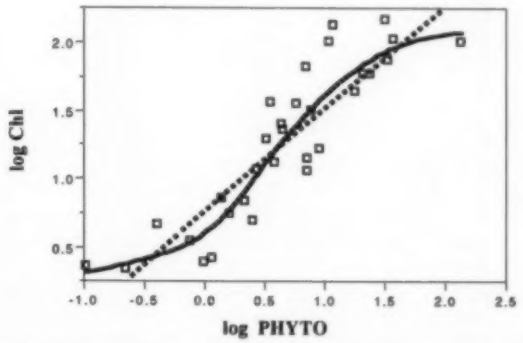

FIG. 9. Plot of $\log_{10}$ Chl (μg/L) versus $\log_{10}$ PHYTO (mg/L) for 31 lakes in Alberta. The solid line was obtained by applying a smoothing spline procedure to the data (r^2 = 0.85) (see Fig. 1 legend); the broken line is the least-squares linear fit through the data (r^2 = 0.79).

to functional categories (see Methods to Test Hypotheses). We found a statistically significant increase in the proportion of filamentous algae with increasing TP concentrations (Fig. 10a), which was accompanied by a concomitant

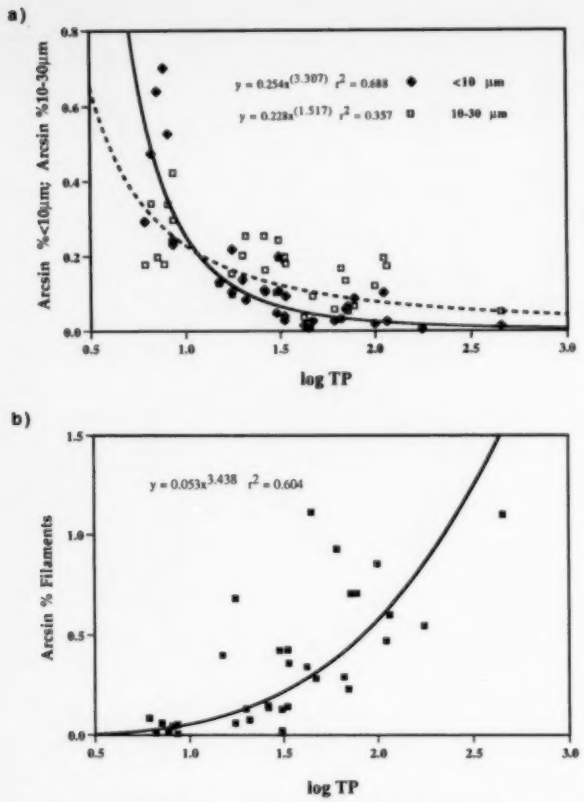

FIG. 10. Proportion of total phytoplankton biomass (arcsin-transformed) plotted as a function of $\log_{10}$ TP (µg/L) for (a) cells with maximum linear dimensions <30 µm (◆, cells <10 µm; □, cells/colonies between 10 and 30 µm) and (b) filamentous algae. Data are from 33 lakes in Ontario (Experimental Lakes Area) and Alberta.

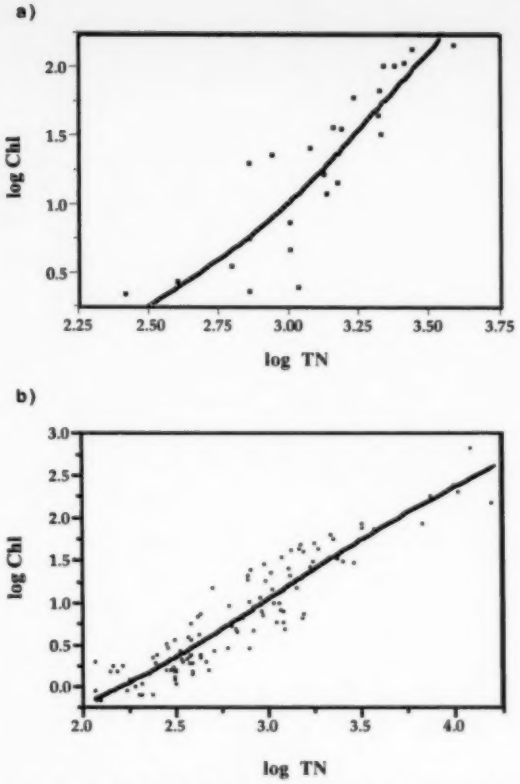

FIG. 11. Plot of $\log_{10}$ Chl *a* (µg/L) versus $\log_{10}$ TN (µg/L) for (a) 31 Alberta lakes and (b) a large database including 119 waterbodies and 152 lake-years. Lines were obtained by applying a smoothing spline procedure to the data (see Fig. 1 legend).

decrease in the proportion of cells <30 µm (including cells in both categories of <10 and 10–30 µm; Fig. 10b). Neither cells nor colonies >30 µm exhibited any systematic variation with TP levels. When the functional groups are presented together in stacked histograms (Fig. 4b), it is obvious that the filaments belonged to the cyanophytes (see Fig. 4a). These observations are therefore consistent with our fourth hypothesis that the grazing impact of herbivores decreases systematically as lakes increase in productivity due to the corresponding increase in the proportion of interfering blue-green filaments.

As a comparison, Watson et al. (1992) conducted a similar study in which algae were assigned to only two functional categories: "edible" algae whose maximum dimensions were <35 µm and "inedible" algae whose dimensions were >35–50 µm. They found that the proportion of the "inedible" fraction increased with lake trophic status, while that of the "edible" fraction decreased. Our results demonstrate that grouping all cells/colonies >35–50 µm as "inedible" may be unwarranted, since only filamentous forms were shown to vary significantly with phosphorus concentrations. The distinction between these "inedible" forms is important because in addition to being inedible, filaments also interfere with grazing activities (Chow-Fraser and Sprules 1986; Hawkins and Lampert 1989). Therefore, separating the "inedible" category into filamentous and nonfilamentous forms should make the functional categories more informative.

Our fifth hypothesis addresses the departure from linearity in the latter portion of the curve. A large body of evidence strongly implicates the limiting effects of TN and/or ambient light availability (Smith 1986; Canfield et al. 1989; Beaver and Crisman 1991; Downing and McCauley 1992) at extremely high TP concentrations (>500 µg/L). When we plotted Chl *a* values against log TN, we found no evidence of levelling off at high TN concentrations for either the smaller subset of 31 lakes (Fig. 11a) or the large dataset (Fig. 11b). It is tempting to infer from this that these hypereutrophic lakes are nitrogen limited; however, without evidence from field kinetic studies, and some information regarding the internal N:P ratios, we do not know whether or not phytoplankton are compensating for low external loading through more efficient nitrogen fixation (Findlay et al. 1994). Nevertheless, the reason that TN appears to be better than TP in predicting Chl *a* in hypereutrophic systems deserves further study.

General Discussion

We have ruled out the first hypothesis that the sigmoidal relationship between TP and Chl *a* could be an artifact of incompatible extraction protocols used in the different

published studies. Although we found that differences in analytical technique contributed significant errors to the measurement of Chl *a* across studies, the nature of these errors did not confound the strong curvilinear relationship between Chl *a* and TP, and thus our accounting for differences in measurement protocol did not change the conclusion that the Chl–TP curve is sigmoidal (Fig. 1). Our study showed that differences in extraction protocol explained as much variation as choice of analytical machine used (i.e., spectrophotometer versus fluorometer; 6% each); neither was substantial compared with the effect of lake origin (81%). Thus, in cross-lake comparisons where the range in TP concentrations spans several orders of magnitude, differences in measurement protocol may be ignored. These results thus validate the approach taken by other investigators who have combined or compared data obtained from different laboratories with different measurement protocols. However, investigators should be careful when comparing data within lakes or among lakes of similar trophic states because depending on the combination of extraction protocol and machine type used, errors may be relatively large.

Although a test of the second hypothesis was inconclusive, we found strong evidence that Chl *a* is a biased surrogate of phytoplankton biomass and that there is an uncoupling between Chl *a* and PHYTO in unproductive lakes (Fig. 6). Part of this uncoupling may be attributed to the fact that the lakes in question tend to be dominated by diatom–chrysophycean assemblages (Stockner et al. 1980; Shortreed and Stockner 1981) that contain large amounts of Chl *c* in addition to Chl *a*. Holm-Hansen et al. (1965) showed that when mixtures of Chl *a* and Chl *c* were present in samples, acid ratios tended to increase, and this resulted in overall higher estimates of Chl *a* than when no other chlorophyll pigments were present. Accordingly, Chl *a* values in these lakes may have been artificially inflated and the initial nonlinearity between TP and Chl *a* may in part reflect the inappropriateness of using Chl *a* as the sole surrogate of algal biomass across a large range of lake productivity. A detailed examination of the relative contribution of the three forms of Chl in natural communities must be undertaken before we can fully evaluate the extent to which Chl *a* and phytoplankton biomass can be used interchangeably across the trophic gradient.

The uncoupling of PHYTO and Chl *a* in this study differs from that of Watson et al. (1992) who found that the Chl:PHYTO ratio in their dataset varied in a nonsystematic fashion with phosphorus (their fig. 2). The primary difference in datasets that led to our respective conclusions is the high Chl:PHYTO ratios corresponding to British Columbia coastal lakes with TP concentrations <5 μg/L in our dataset (Fig. 6a), which had no counterpart in Watson et al.'s (1992) dataset. Without a corresponding breakdown of taxonomic or size categories of phytoplankton in these coastal lakes, we cannot comment on possible reasons for these differences.

We tested both our third and fourth hypotheses to explain the initial nonlinearity in the TP–Chl curve and found that both were upheld. Our third hypothesis was supported by the observation that the proportion of TBAP increased linearly with TP throughout most of the TP concentrations tested (<200 μg/L; Fig. 7a) and the concomitant observation that Chl *a* varied directly with the proportion of TBAP in the TP fraction (Fig. 7b). These observations suggest that Chl in unproductive systems is largely responding to the amount of biologically available phosphorus in the water column, and since this is a relatively small proportion of the TP pool in oligotrophic lakes, TP would tend to overestimate algal biomass. Our fourth hypothesis was supported by a significant increase in the proportion of interfering blue-green algae with increasing TP concentrations (Fig. 10a). We therefore suggest that grazers in oligotrophic lakes can exert a greater impact on the algae compared with grazers in more productive systems because phytoplankton communities associated with the former are filament-free. Unfortunately, we could not determine the extent to which either hypothesis applies or if they both contribute equally to the initial nonlinearity.

Our final hypothesis was used to explain the departure from linearity at extremely high concentrations of TP. The fact that Chl concentrations did not plateau at high TN concentrations is consistent with the hypothesis that TN is more limiting than TP in these hypereutrophic lakes (Fig. 11). However, no conclusions regarding nitrogen limitation should be made without further studies including field kinetic studies and an investigation of the internal N:P ratios.

This study has provided evidence that the sigmoidal relationship between TP and Chl *a* could be attributed to several sets of ecological interactions between adjacent trophic levels at the bottom of the aquatic foodweb. We hope that more hypotheses will be formulated in future studies to uncover the underlying mechanisms that lead to nonlinearity in the TP–Chl *a* relationship. It is important that limnologists continue to examine ecological functions over the entire productivity gradient because theories that provide a unifying framework cannot be advanced unless investigations include a global perspective.

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Appendix

TABLE A. Mean seasonal nutrient (TP, TN; μg/L), Chl *a* (μg/L), and phytoplankton biomass (mg/L) concentrations for 33 lakes in Ontario (Experimental Lakes Area) and Alberta. COL, colonies; FIL, filaments; Total, sum of all functional or taxonomic groups. Slight discrepancies are due to rounding errors. Means are calculated from data collected at least monthly between May and September inclusive. n/c, not counted; n/a, not available.

					Algal biomass											
Lake	Year	TP	TN	Chl	<10 μm	10–30 μm	>30 μm	COL	FIL	Cyanophyte	Chlorophyte	Chrysophyte	Diatoms	Cryptophyte	Dinoflagellate	Total
L382	1980	6.1	386	3.6	0.187	0.115	0.296	n/c	0.053	0.056	0.117	0.350	0.038	0.054	0.035	0.651
L373	1983	6.6	239	0.8	0.187	0.137	0.084	n/c	0.004	0.011	0.038	0.229	0.028	0.031	0.074	0.412
L224	1974	7.1	237	2.2	0.319	0.105	0.080	n/c	0.031	0.040	0.043	0.375	0.019	0.016	0.042	0.535
L239	1974	7.7	312	3.6	0.300	0.083	0.074	n/c	0.009	0.023	0.034	0.318	0.020	0.031	0.040	0.466
L226S	1974	8.1	418	6.9	0.988	0.653	0.240	n/c	0.086	0.116	0.057	1.201	0.091	0.071	0.432	1.967
L239	1982	8.6	281	3.2	0.203	0.247	0.391	n/c	0.005	0.019	0.025	0.450	0.178	0.047	0.128	0.846
L382	1983	8.6	380	2.4	0.208	0.372	0.283	n/c	0.045	0.072	0.107	0.295	0.145	0.070	0.220	0.908
Marie	1981	15.0	683	4.6	0.197	0.193	0.469	0.066	0.587	0.741	0.108	0.191	0.323	0.183	0.135	1.511
Bourque	1981	17.5	640	5.3	1.334	1.929	8.428	0.199	0.725	0.935	0.575	1.326	1.945	0.255	8.557	12.615
L226N	1974	17.6	501	15.2	1.169	0.533	0.293	n/c	3.412	3.429	0.136	1.410	0.147	0.086	0.026	5.407
Touchwood	1986	20.0	831	4.8	0.098	0.144	0.380	0.004	0.092	0.124	0.013	0.055	0.302	0.236	0.028	0.718
Ethel	1984	20.8	727	6.7	0.152	0.451	0.738	0.329	0.132	0.453	0.074	0.214	0.712	0.154	0.196	1.802
Island	1984	26.1	1160	8.3	0.168	0.381	0.728	0.024	0.221	0.238	0.089	0.113	0.741	0.198	0.143	1.522
Moore	1981	26.3	935	7.1	3.016	4.081	12.189	0.131	2.529	2.223	1.457	2.056	8.449	2.725	4.943	21.945
N. Buck	1986	30.2	1059	14.5	0.190	0.401	1.484	0.200	1.592	2.229	0.679	0.164	0.496	0.280	0.544	3.867
Bonnie	1983	31.0	1203	6.0	0.138	0.248	0.724	0.006	0.160	0.203	0.145	0.098	0.250	0.286	0.098	1.276
Minnie	1986	31.0	1117	5.9	0.276	0.336	0.663	0.096	0.029	0.113	0.596	0.212	0.158	0.155	0.304	1.401
Beaver	1986	33.2	1146	9.9	0.090	0.584	1.672	0.336	0.433	0.740	0.365	0.103	1.236	0.277	0.457	3.114
Battle	1984	33.3	611	9.4	0.042	0.205	0.357	0.006	0.431	0.438	0.008	0.023	0.308	0.164	0.103	1.041
Skeleton N.	1986	33.8	n/a	9.1	0.166	0.314	0.585	0.080	0.617	0.800	0.151	0.096	0.299	0.243	0.320	1.761
Buck	1984	42.2	772	20.8	0.159	0.385	6.143	0.127	3.412	3.463	0.131	0.158	6.080	0.306	0.090	10.227
Pinehurst	1986	44.8	1205	14.2	0.072	0.227	0.625	0.001	8.004	7.479	0.017	0.237	0.081	0.316	0.426	8.929
Skeleton S.	1986	47.0	n/a	24.7	0.127	0.444	2.233	0.606	1.306	2.202	0.227	0.086	1.346	0.284	0.892	4.716
Iosegun	1983	61.0	950	29.2	0.161	0.350	0.602	0.069	4.761	3.388	0.224	0.074	0.538	1.190	0.149	5.943
Baptiste S.	1984	66.3	1787	49.8	0.274	1.373	3.824	0.433	2.343	2.746	0.312	0.056	1.587	0.428	3.127	8.247
Baptiste N.	1984	70.0	1332	41.7	0.611	1.389	5.655	0.321	2.332	2.652	0.315	0.180	2.283	0.513	4.066	10.308
Tucker	1982	72.1	1312	21.0	0.366	0.292	1.145	0.117	3.559	4.204	0.372	0.132	0.362	0.699	0.110	5.480
Nakamun	1984	77.8	1742	30.6	0.426	0.324	0.792	0.153	3.147	3.779	0.145	0.319	0.782	0.236	0.064	4.842
Sturgeon E.	1984	99.7	1493	48.1	0.423	1.353	1.176	0.294	6.865	7.978	0.178	0.125	1.555	0.217	0.101	10.111
Sturgeon W.	1984	111.1	1546	40.9	1.111	2.089	1.651	1.022	4.906	6.402	1.920	0.256	2.345	0.266	0.185	10.779
Isle	1984	115.5	1466	43.3	0.153	1.017	1.253	0.129	3.329	4.028	0.039	0.020	1.100	0.262	0.071	5.881
Coal	1984	175.9	1856	43.8	0.054	0.125	5.557	0.386	6.646	7.032	0.039	0.007	5.483	0.191	0.017	12.768
Driedmeat	1984	452.4	3202	86.6	0.427	1.632	0.971	0.395	27.979	28.363	0.608	0.290	1.816	0.327	0.000	31.404

Maturation of White Sucker, *Catostomus commersoni*, Populations in Ontario

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Chen, Y., and H.H. Harvey. 1994. Maturation of white sucker, *Catostomus commersoni*, populations in Ontario. Can. J. Fish. Aquat. Sci. 51: 2066–2076.

White sucker, *Catostomus commersoni*, were sampled from 13 Ontario lakes. Two growth parameters (growth rate in length as dL/dt at age t_0 and age at maximum growth rate in weight as dW/dt) were estimated by fitting length- and weight-at-age data to the two reparameterized von Bertalanffy growth functions for each of 13 populations. Two maturation parameters (length and age at 50% maturity, ML and MA) were estimated using the linear regression method for both sexes. Two regression approaches were used in analyzing experimental data: a traditional geometric means (GM) method and a two-step procedure of robust regression analysis which includes applying the least median squares method to identify possible outliers and applying the GM method to data exclusive of the defined outliers. Great among-population variation in the estimated growth and maturation parameters was observed for both female and male white sucker. White sucker growing faster at young ages tended to mature earlier. High population density might result in white sucker having slower growth rates in their earlier ages and larger MA's. This study indicated that maturation of white sucker is food dependent, and early growth rate is an important factor in determining the timing of maturity.

On a prélevé des meuniers noirs, *Catostomus commersoni*, dans 13 lacs de l'Ontario. Deux paramètres de croissance (taux de croissance en longueur (dL/dt) à l'âge t_0 et l'âge auquel le taux de croissance en poids (dW/dt) est maximal) ont été estimés en ajustant les données de poids et le longueur aux différents âges aux deux fonctions de croissance de von Bertalanffy reparamétrisées, pour chacune des 13 populations. Deux paramètres de maturation (longueur et âge à mi-chemin de la maturité, ML et MA) ont été estimés à l'aide de la méthode de régression linéaire pour les deux sexes. On a eu recours à deux méthodes de régression pour analyser les données expérimentales : une méthode classique utilisant les moyennes géométriques (GM) et une procédure en deux temps d'analyse de régression robuste qui inclut l'application de la méthode de la médiane quadratique minimale pour détecter les valeurs aberrantes possibles et l'application de la méthode de la moyenne géométrique aux données autres que les valeurs aberrantes trouvées. On a constaté entre les populations de grandes variations dans les paramètres de croissance et de maturation estimés, tant pour les mâles que pour les femelles de l'espèce. Les meuniers noirs qui ont une croissance plus rapide lorsqu'ils sont jeunes ont tendance à atteindre la maturité plus tôt. Une forte densité de population pourrait avoir comme conséquence que les meuniers noirs aient une croissance plus lente dans leurs premières années et des MA plus élevés. L'étude indique que la maturation des meuniers noirs est fonction de la nourriture, et que le taux de croissance en bas âge est un facteur important dans la détermination du moment où sera atteinte la maturité.

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White sucker, *Catostomus commersoni*, is a widely distributed and very abundant species that may comprise half or more of the total fish biomass in some Ontario Lakes (Scott and Crossman 1973; Lalancette 1977; Fraser 1981; Harvey 1978, 1982; Trippel and Harvey 1987). Although great variation has been observed in age and length at maturity among some populations (Beamish 1970; Trippel and Harvey 1989, 1991), few reports identify the abiotic and biotic environmental variables related to the maturation of white sucker. Trippel and Harvey (1989) reported that the onset of maturation was energy dependent. However, this result was based on five populations only, and the correlation between frequency of refraining from reproduction among five white sucker populations versus density of chironomid forage was not significant ($r = -0.72$ and $P = 0.17$). Lake pH values have been reported to relate to white sucker maturation (e.g., Beamish 1970; Trippel and Harvey 1989). The relationship between growth and maturation of white suckers has been examined in some studies. Trippel and Harvey (1989) reported that a critical minimum annual growth, diminishing with age, may be necessary to initiate maturation. However, the growth parameter used in their study was size at age 4, which was appropriate for their late-maturing Gullfeather Lake population, but which is older than average age at maturation (i.e., 3–4 yr old; Scott and Crossman 1973) of white sucker. White sucker maturation has not been related to early growth rate in length (or weight). Maturation of white sucker has been reported to influence subsequent growth (Lalancette 1977; Quinn and Ross 1975; Trippel and Harvey 1989), but no quantitative

TABLE 1. Lake geographical location, morphometric characteristics, white sucker population estimates, estimates for densities of chironomid and ephemeropteran larvae, sampling year for white sucker and benthos, and white sucker sample sizes in 13 lakes.

Lake	Lat.	Long.	S^a	H^b	P^c	Chiro[d]	Eph[e]	pH	Year (N)
Bentshoe	45°02′	78°56′	16.8	9	NA	3 933	694.5	5.6	1981 (126)
Blue Chalk	45°12′	78°56′	49.4	23	3 224	8 140	1896.0	6.5	1979 (71)
Buck	45°23′	79°00′	40.3	30	676	18 945	101.9	6.7	1980 (67)
Chub	45°13′	78°59′	32.2	27	2 529	3 220	28.5	5.6	1981 (93)
Crosson	45°05′	79°02′	56.8	25	764	17 176	140.9	5.9	1981 (108)
Dickie	45°09′	79°05′	92.0	12	7 670	3 589	7.4	5.7	1979 (99)
George	46°02′	81°24′	147.0	37	764	5 500	0.0	5.0	1982 (79)
Gullfeather	45°06′	79°01′	65.9	13	11 329	1 814	3.0	5.5	1982 (77)
King	45°53′	79°30′	29.0	6	870	2 439	1.8	5.0	1983 (78)
McQuaby	46°02′	79°34′	224.2	14	NA	NA	NA	5.9	1988 (107)
Red Chalk	45°11′	78°57′	56.9	38	4 206	4 122	7.1	6.4	1981 (111)
Secord Pond	43°58′	79°16′	1.5	6	NA	NA	NA	8.0	1988 (150)
Solitaire	45°23′	79°00′	124.0	31	2 869	6 751	0.0	6.3	1980 (118)

[a]Lake area (ha).
[b]Lake maximum depth (m).
[c]Estimates of white sucker population size.
[d]Density estimates for chironomid larvae (no./m^2)
[e]Density estimates for ephemeropteran larvae (no./m^2).

relationship has been determined. All previous studies on the relationship between growth and maturation of white sucker were based on small numbers of populations ($N \leq 5$).

The objective of this study was to investigate the variation in maturation of female and male white sucker among 13 populations with respect to growth and environmental variables. The following hypotheses were tested : (I) white sucker growing faster in their earlier years (i.e., prior to maturity) mature earlier than those having a slower growth rate, (II) age at maturity of white sucker is related to age at maximum growth rate in weight, (III) white sucker attaining maturity at an earlier age have smaller maximum attainable length, and (IV) maturation in white sucker is associated with abiotic and biotic environmental variables. The testing of these hypotheses would enable us to understand better how maturation of white sucker related to growth and what environmental variables potentially influence maturation.

Materials and Methods

A total of 1206 white sucker were collected from 13 Ontario lakes, 1979–88 (Table 1). Fish fork length was measured to the nearest 0.1 cm and total weight to the nearest 5 g. The first pectoral fin ray was removed from each fish for the purpose of age determination and back-calculation of growth. Sex determination was based on macroscopic inspection of the gonads or on a unique feature of mature males: horny tubercles on the anal fin ray. Stages of sexual maturation were assigned based on the criteria of Trippel (1984). The procedures in calculating length- and weight-at-age data were detailed by Chen (1991).

The von Bertalanffy growth function (VBGF) is the most popular growth function in fisheries and is commonly expressed as

$$L_t = L_\infty(1 - e^{-K(t-t_0)})$$

in length and

$$W_t = W_\infty(1 - e^{-K^W(t-t_0^W)})^3$$

in weight (superscript W associated with K and t_0 implies that the parameters were estimated from weight-at-age data) where L_t and W_t are the length and weight at age t, K and K^W are the length- and weight-based Brody growth parameters, t_0 and t_0^W are the ages at length and weight of 0, and L_∞ and W_∞ are the maximum attainable length and weight (Ricker 1975; Moreau 1987). Two growth parameters have been proposed: (1) the growth rate in length as dL/dt at age t_0 (ω, Gallucci and Quinn 1979) and (2) the age at maximum growth rate in weight as dW/dt (IA, Jensen 1985). They are calculated as

$$\omega = KL_\infty$$

$$\mathrm{IA} = \frac{Ln(3)}{K^W} + t_0^W$$

where all parameters are the same as those in VBGF's.

By introducing ω and IA into the length- and weight-based VBGF's, respectively, VBGF's are reparameterized as

$$L_t = \frac{\omega}{K}(1 - e^{-K(t-t_0)})$$

$$W_t = W_\infty\left(1 - \frac{e^{-K^W(t-\mathrm{IA})}}{3}\right)^3.$$

For each white sucker population, length- and weight-at-age data were fitted to these two equations, respectively, to estimate ω, IA, and their associated uncertainties using the nonlinear least squares method (Marquardt method, PROC NLIN, SAS 1987).

From the plot of dL/dt against t (i.e., age), it is easy to show that ω is the growth rate as dL/dt at the hypothetical age t_0. For a well-estimated VBGF, t_0 usually is a negative value (as fish have length at age 0). Thus, ω may not exist in a practical sense. However, it is more or less related to the growth rate of young fish as noted by Kingsley (1980) and Moreau (1987). IA represents the age at maximum growth rate in weight as dW/dt, and thus represents a definable stage in the growth process. It is easy to show that IA is an age at which length is two-thirds L_∞ if the relationship between weight and length is isometric. IA has been reported

to be close to the age at 50% maturity for lake whitefish, *Coregonus clupeaformis* (Jensen 1985).

For each population, fish were grouped into 1-cm length intervals, and the proportion of fishes having attained maturity was calculated for each length interval. The calculated proportions were transformed using $\sin^{-1}\sqrt{}$ (arcsine square root; ASR) and then regressed against the respective midpoint values of the length intervals using a simple linear model with the least squares (LS) method. Significant regression equations were solved to estimate the length at which half of the fishes were mature (ML, Trippel and Harvey 1991). ML determined from the regression was then converted to age at 50% maturity (MA) by the corresponding length-based VBGF (Trippel and Harvey 1991). MA and ML were calculated for female and male separately for each population.

Some abiotic and biotic environmental variables were selected for assessment of their potential effects on white sucker maturation, including lake area and maximum depth, lake pH, abundance of benthic organisms, and white sucker population density. Selection of these variables was dependent on their ecological importance, descriptive ability, and conformity with previously published analyses (see Beamish 1970; Trippel and Harvey 1987, 1989, 1991).

Sizes of six white sucker populations (i.e., Crosson, Dickie, George, Gullfeather, King, and Red Chalk lakes) were estimated using the Petersen mark–recapture method as defined by Ricker (1975). Sizes of four other populations were estimated using the catch-per-unit-effort method defined by Trippel (1984). No estimates of population size were obtained for three lakes due to the difficulty in standardizing fishing effort (Table 1). The population density was derived as population size divided by lake area. White sucker is considered a secondary consumer feeding on benthic invertebrates and microcrustaceans (Eder and Carlson 1977; Trippel and Harvey 1987; Ahlgren 1990). Chironomid and ephemeropteran larvae have been identified as their most important food organisms (Johnson 1977; Trippel and Harvey 1987). For a particular lake, benthos was sampled and processed at the same season in the year when white sucker were collected in 11 of the 13 lakes based on the procedures of Allison and Harvey (1981). For each lake, chironomids and ephemeropterans were quantified from 60 cores taken along six transect lines passing through the three depth zones based on the thermocline. The abundances of chironomid and ephemeropteran larvae were represented by average densities over the whole lake.

The relationships between maturation parameters of white sucker versus environmental variables were investigated using regression analysis. The most commonly used estimation methods for regression analysis in fisheries studies are LS and geometric mean (GM). The LS method is often used when the independent variables are free of the errors or have much smaller errors compared with the dependent variable. Use of the GM method is suggested when the independent variables are subject to errors (Ricker 1975; McArdle 1988). However, both methods can fail in estimation due to any of the following reasons (Weisberg 1980): (1) heterogeneity of error variance, (2) nonnormality of errors, (3) inadequacy of the hypothesized model, or existence of outliers. The first problems often can be overcome by an appropriate variance-stabilizing transformation or by using a generalized LS method (e.g., a weighted LS, Sen and Srivastava 1990). The third problem can be overcome by changing the regression model. The failure of a regression model due to the existence of outliers can be remedied by excluding outliers from the regression analysis. However, the traditional LS-based outliers diagnostics methods usually fail when there are multiple outliers in data (Rousseeuw and Leroy 1987; Hawkins 1993; Chen et al. 1994). Because the LS-based methods are sensitive to outliers, the estimated regression lines are pulled towards outliers. Thus, outliers do not necessarily have large residuals, and may not be identified in residual analyses. Data obtained in fisheries studies are usually subject to various errors such as measurement error and heterogeneous environmental variation. The existence of outliers can be expected in regression analyses of fisheries data (Chen et al. 1994; Chen and Paloheimo 1994). In this study, data for white sucker and environments may be subject to various sources of error. Thus, the LS or GM method alone may not be a good estimation method.

A robust regression method, least median squares (LMS), was developed to minimize the effect of outliers on estimation of regression parameters (Rousseeuw and Leroy 1987). Instead of minimizing residual sum of squares like the LS estimator, the LMS minimizes the median of squared residuals (see Chen et al. 1994). Because of its insensitivity to outliers, the outliers are usually far away from the LMS-estimated regression line; thus, outliers can be detected readily by a residual analysis. Based on an extensive simulation study, Rousseeuw and Leroy (1987) developed a criterion to judge whether a data point is an outlier in a regression analysis (see Chen et al. 1994). Although the LMS estimator is robust with respect to outliers in both the Y and X variables, this method has poor large-sample statistical efficiencies and does not provide variance estimates analogous to those of the least squares for the estimated regression parameters. To solve the problem, a two-step procedure is proposed for regression analysis: identify possible outliers by the LMS method and then apply a more traditional approach (e.g., LS or GM) to the data with the LMS-defined outliers being excluded. Many studies have shown that this approach derives similar estimates for regression parameters as the traditional methods (LS or GM) when there are no outliers, but provides the parameter estimates with much smaller biases compared with the traditional methods when there are outliers (see Rousseeuw and Leroy 1987; Dallal 1991; Dallal and Rousseeuw 1992; Hawkins 1993; Chen et al. 1994; Chen and Paloheimo 1994).

In this study, the two-step regression procedure was used: apply the LMS to identify possible outliers and then use a traditional method to analyze data with the LMS-defined outliers excluded. Because of the errors associated with the estimates of white sucker maturation parameters and environmental variables, the GM method was used as the traditional method in the two-step analysis. It is important to recognize that identification must be done relative to a specific regression model (Weisberg 1980; Rousseeuw and Leroy 1987). A population identified as an outlier in the LMS analysis of two variables is not necessarily an outlier if the regression variables are changed.

Results

The LS-estimated ω and IA differed significantly from 0 for all populations ($P < 0.05$, Tables 2 and 3). Females had larger maximum attainable lengths (L_∞'s) than males within

TABLE 2. Nonlinear least squares estimates of parameters of the length-based von Bertalanffy growth function for white sucker.

Lake	L_∞		K		t_0		ω	
	Esti[a]	SE[b]	Esti	SE	Esti	SE	Esti	SE
				(a) Females				
Bentshoe	41.2	0.91	0.26	0.016	−0.16	0.072	10.7	0.59
Blue Chalk	54.4	4.59	0.16	0.024	0.19	0.109	8.6	0.60
Buck	46.4	0.79	0.35	0.029	−0.09	0.119	16.4	1.13
Chub	46.9	1.53	0.32	0.023	−0.22	0.051	15.0	0.61
Crosson	44.6	1.26	0.36	0.036	0.10	0.121	16.2	1.25
Dickie	43.0	0.53	0.19	0.006	−0.76	0.044	8.0	0.15
George	43.7	1.08	0.35	0.026	0.13	0.068	15.3	1.25
Gullfeather	40.5	0.46	0.21	0.007	−0.43	0.043	8.7	0.17
King	38.4	0.39	0.39	0.039	−1.23	0.270	14.8	1.38
McQuaby	44.8	1.40	0.57	0.071	0.23	0.097	25.6	2.50
Red Chalk	44.9	0.89	0.25	0.016	−0.54	0.108	11.0	0.52
Secord Pond	37.9	2.76	0.42	0.085	−0.47	0.168	15.8	2.08
Solitaire	50.2	2.91	0.26	0.034	−0.44	0.121	11.7	1.38
				(b) Males				
Bentshoe	40.9	0.81	0.27	0.011	−0.15	0.061	10.8	0.53
Blue Chalk	40.0	1.53	0.24	0.028	0.04	0.160	8.7	0.70
Buck	41.4	0.54	0.46	0.029	−0.04	0.073	19.2	1.00
Chub	46.4	3.76	0.29	0.048	−0.27	0.103	13.5	1.17
Crosson	40.8	0.59	0.42	0.021	0.19	0.049	17.1	0.64
Dickie	39.3	0.76	0.22	0.014	−1.55	0.141	8.5	0.41
George	43.1	1.11	0.37	0.028	−0.03	0.034	15.9	0.95
Gullfeather	33.1	0.63	0.33	0.019	−0.22	0.065	10.9	0.44
King	35.0	0.32	0.47	0.053	−1.05	0.282	16.3	1.77
McQuaby	41.5	0.57	0.66	0.054	−0.26	0.096	27.2	1.91
Red Chalk	41.3	0.72	0.28	0.017	−0.58	0.103	11.4	0.53
Secord Pond	28.3	0.03	0.71	0.003	−0.12	0.003	20.0	0.07
Solitaire	50.0	2.86	0.29	0.038	−0.40	0.110	14.4	1.46

[a]Estimate of the parameter.
[b]Associated standard error.

each population. Among-population variabilities were much larger than the variation between female and male white sucker within populations. Males tended to have larger ω's than females in all populations except that of Chub Lake (Table 2). Female white sucker tended to have larger IA's than males in all populations (Table 3).

Significant LS regression equations existed between the ASR-transformed proportion of mature fishes and the corresponding fork length for female and male white sucker in all 13 populations (Table 4). This relationship differed significantly in slope and intercept among populations for both females and males (ANCOVA, $P < 0.001$, PROC GLM, SAS 1987). ML ranged from 20.3 to 36.3 cm (1.8-fold difference) for females and from 19.6 to 29.1 cm (1.5-fold) for males (both minima Solitaire Lake and maxima McQuaby Lake). MA varied from 1.4 yr of age to 7.5 yr (5.4-fold difference) for females and from 1.2 to 6.0 yr (5.0-fold) for male white sucker (both minima Secord Pond and maxima Gullfeather Lake). Female white sucker matured at a larger size and older age than males in all 13 populations (Table 4). MA and ML of females were highly correlated with those of males, respectively. Length at maturity as a fraction of maximum attainable length (ML/L_∞) varied greatly: 0.40 in the Solitaire population to 0.83 in the Bentshoe population for females and from 0.39 in the Solitaire population to 0.87 in the Gullfeather population for males (Table 4). However, this ratio was close within populations (Table 4). The GM-estimated regression equation was

$$ML/L_\infty \text{ for females} = -0.17(0.122) + 1.30(0.187)\,(ML/L_\infty \text{ for males}),\ r = 0.83,\ P = 0.001$$

where numbers inside parentheses are standard errors for the estimated parameters, r is the correlation coefficient, and P is the attained significance level. There were no significant differences between the estimated intercept and 0 and between the estimated slope and 1. Thus, an isometric relationship existed between female and male white sucker (Fig. 1).

Logarithm MA was negatively and significantly correlated with logarithm ω for both sexes of white sucker (Fig. 2). The GM regression equations were estimated for females and males, respectively, as

$$\log(MA) = 3.61(0.455) - 2.76(0.405)\log(\omega),\ r = -0.62,\ P = 0.003$$

$$\log(MA) = 1.16(0.339) - 1.52(0.293)\log(\omega),\ r = -0.77,\ P = 0.002$$

where log = $\log_{10}$. Two populations, McQuaby and Secord Pond, were identified as outliers for female white sucker in the LMS analysis. No outliers were identified for males (Fig. 2). The LMS-based reweighted GM regression equation

TABLE 3. Nonlinear least squares estimates of parameters of the weight-based von Bertalanffy growth function for white sucker.

	W_∞		K^W		t_0^W		IA	
Lake	Esti[a]	SE[b]	Esti	SE	Esti	SE	Esti	SE
				(a) Females				
Bentshoe	769.7	34.0	0.33	0.023	0.31	0.164	3.7	1.51
Blue Chalk	1413.4	77.5	0.24	0.015	0.89	0.121	5.5	0.18
Buck	1806.3	147.6	0.21	0.031	−1.26	0.536	3.9	0.27
Chub	2067.8	210.8	0.26	0.024	−0.29	0.121	3.9	0.27
Crosson	1486.8	157.7	0.26	0.054	−0.59	0.605	3.6	0.38
Dickie	851.0	16.5	0.20	0.004	−1.19	0.007	4.2	0.07
George	1418.5	104.0	0.26	0.026	−0.30	0.221	3.9	0.72
Gullfeather	662.2	15.6	0.24	0.009	−0.84	0.102	3.7	0.08
King	800.1	22.0	0.33	0.042	−2.12	0.512	1.2	0.54
McQuaby	1218.9	67.9	0.74	0.112	0.60	0.216	2.1	0.10
Red Chalk	1184.3	32.2	0.30	0.015	0.11	0.141	3.8	0.08
Secord Pond	599.8	162.6	0.36	0.096	−0.76	0.317	2.3	0.55
Solitaire	1521.6	123.1	0.37	0.040	0.20	0.148	3.2	0.43
				(b) Males				
Bentshoe	758.9	31.1	0.32	0.018	0.28	0.142	3.7	0.27
Blue Chalk	730.7	54.3	0.34	0.039	1.00	0.237	4.2	0.18
Buck	1049.4	47.9	0.40	0.043	−0.22	0.251	2.5	0.13
Chub	1257.0	297.2	0.35	0.073	0.01	0.208	3.1	0.47
Crosson	1002.9	53.9	0.36	0.038	−0.30	0.248	2.7	0.14
Dickie	740.8	39.0	0.21	0.017	−2.32	0.265	3.0	0.19
George	1401.2	98.2	0.26	0.017	−0.25	0.212	3.9	0.13
Gullfeather	439.1	25.0	0.35	0.029	0.00	0.160	3.2	0.13
King	608.0	15.1	0.42	0.061	−1.75	0.532	0.9	0.34
McQuaby	1041.2	39.5	0.68	0.067	−0.16	0.147	1.4	0.06
Red Chalk	941.4	27.6	0.31	0.017	−0.11	0.147	3.4	0.09
Secord Pond	225.7	1.1	0.72	0.062	−0.19	0.009	1.3	0.01
Solitaire	1512.3	90.8	0.40	0.036	0.20	0.121	2.9	0.16

[a]Estimate of the parameter.
[b]Associated standard error.

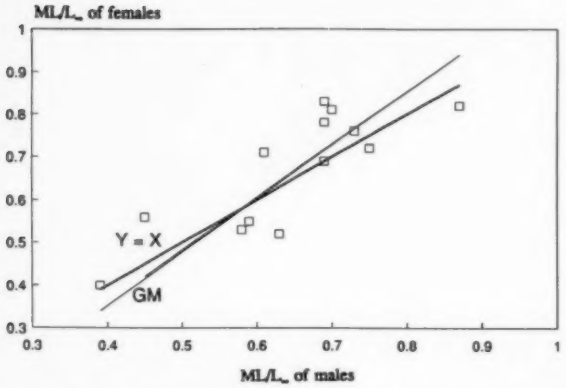

FIG. 1. Length at maturity as a fraction of maximum attainable length (ML/L_∞), female versus male white sucker for 13 populations.

was estimated for females as

$$\log(MA) = 3.51(0.171) - 2.73(0.457)\log(\omega),\ r = -0.73,\ P = 0.001.$$

MA was not significantly related to IA for female white sucker (Fig. 3), but the differences between MA and IA were negatively and significantly related to the logarithm of the density of lake chironomid larvae (Fig. 3). The GM regression was estimated as

$$MA - IA = 23.81(6.028) - 6.28(1.615)\log(\text{density of chironomids}),\ r = -0.65,\ P = 0.029.$$

There were a significant relationship between IA and MA for male white sucker, and the slope of this regression did not differ significantly from 1 (Fig. 4). No outliers were defined in the LMS analysis. The GM-estimated regression equation was

$$\log(MA) = -0.03(0.110) + 1.103(0.244)\log(IA),\ r = 0.68,\ P = 0.009.$$

The regression equation between the difference of MA and IA (i.e., MA − IA) versus log(density of chironomids) was not significant for male white sucker.

The regression equations between $\log(L_\infty)$ and log(MA) were not significant for both females and males. The LS-estimated equations (because r^2 was too small, we used the LS-estimated equations instead of the GM-estimated equations) for females and males, respectively, were

$$\log(L_\infty) = 1.65(0.031) - 0.006(0.054)\log(MA),\ r = 0.1,\ P = 0.914$$

TABLE 4. Estimation of length at 50% maturity (ML), age at 50% maturity (MA), and the ratio of ML against von Bertalanffy's maximum attainable length (L_∞) for female (F) and male (M) white sucker (r^2 = coefficient of determination).

		Intercept		Slope[a]					
Lake		Esti	SE	Esti	SE	r^2	MA	ML	Ratio[b]
Bentshoe	F	−139.7	20.18	5.41	0.548	0.92	6.7	34.1	0.83
	M	−120.1	36.50	5.84	1.235	0.74	4.3	28.3	0.69
Blue	F	−395.8	77.86	15.65	3.041	0.83	4.8	28.2	0.52
Chalk	M	−396.4	81.99	17.59	3.712	0.84	4.2	25.1	0.63
Buck	F	−219.4	50.31	10.78	1.937	0.86	2.0	24.5	0.53
	M	−150.2	36.97	8.16	1.454	0.84	1.8	23.9	0.58
Chub	F	−92.8	40.05	5.29	1.241	0.72	2.3	26.0	0.56
	M	−160.8	27.65	9.10	1.308	0.89	1.8	21.0	0.45
Crosson	F	−195.7	35.78	7.87	1.211	0.86	3.4	30.6	0.69
	M	−98.5	33.91	5.10	1.219	0.69	2.9	28.1	0.69
Dickie	F	−288.0	25.52	9.90	1.461	0.63	7.4	33.6	0.78
	M	−409.5	54.55	16.67	1.933	0.67	3.9	27.3	0.69
George	F	−199.4	32.57	7.83	0.994	0.91	3.7	31.2	0.71
	M	−88.9	18.17	5.05	0.616	0.92	2.8	26.5	0.61
Gullfeather	F	−144.5	20.52	5.73	0.585	0.92	7.5	33.1	0.82
	M	−120.2	12.26	5.73	0.382	0.97	6.0	28.8	0.87
King	F	−345.4	68.45	13.38	2.316	0.84	2.4	29.2	0.76
	M	−249.4	64.30	11.52	2.541	0.74	1.7	25.6	0.73
McQuaby	F	−263.6	75.94	8.51	2.046	0.62	3.1	36.3	0.81
	M	−242.7	85.80	9.89	2.678	0.68	1.6	29.1	0.70
Red	F	−88.5	12.27	4.14	0.420	0.93	4.6	32.2	0.72
Chalk	M	−67.9	9.44	3.65	0.317	0.94	4.4	30.9	0.75
Secord	F	−219.8	99.13	12.76	4.381	0.55	1.4	20.8	0.55
Pond	M	−60.8	25.44	6.33	1.180	0.63	1.2	16.7	0.59
Solitaire	F	−92.3	20.89	6.77	0.924	0.88	1.6	20.3	0.40
	M	−111.2	24.33	7.98	1.118	0.88	1.5	19.6	0.39

[a]Regression of fish fork length against the corresponding $\sin^{-1}\sqrt{}$-transformed proportion of mature fish. All resultant regression equations were significant at the level of 0.001.
[b]=ML/L_∞.

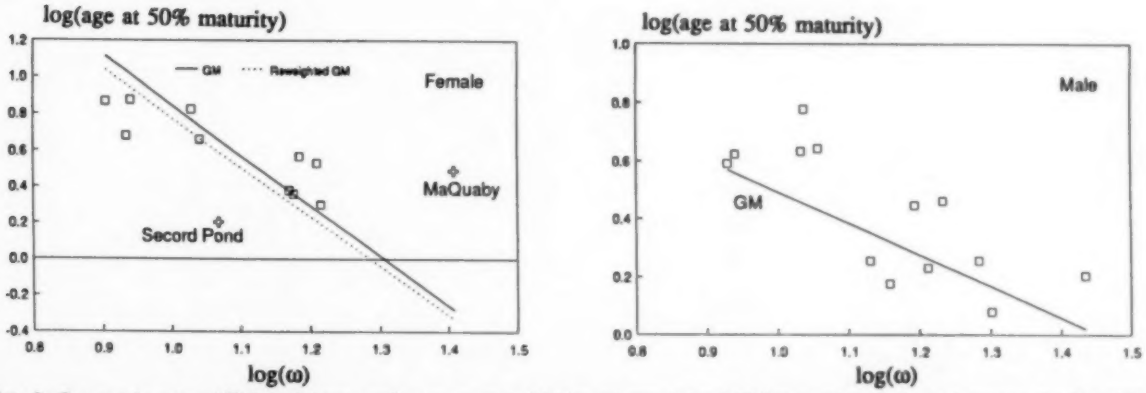

FIG. 2. Geometric mean (GM) and least median squares (LMS) based reweighted GM regression analyses between the logarithm age at 50% maturity (MA) and Gallucci and Quinn's ω for female and male white sucker. The populations labelled are the outliers identified in the LMS analysis.

$$\log(L_\infty) = 1.61(0.045) - 0.014(0.092)\log(\text{MA}), \quad r = 0.1,\ P = 0.882.$$

The King Lake and Secord Pond populations were identified as outliers in the LMS analysis of $\log(L_\infty)$ versus log(MA) for both female and male white sucker (Fig. 5). Blue Chalk also was identified as an outlier population for females. The

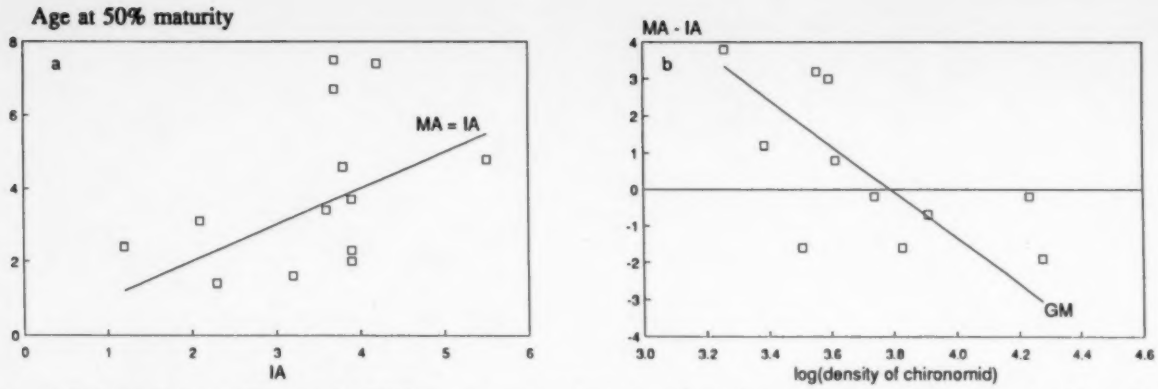

FIG. 3. (Left panel) Age at 50% maturity (MA) versus age at maximum growth rate in weight as *dW/dt* (IA) for female white sucker; (right panel) geometric mean (GM) regression analyses between the difference of MA and IA (MA − IA) versus the logarithm of chironomid larvae for female white sucker.

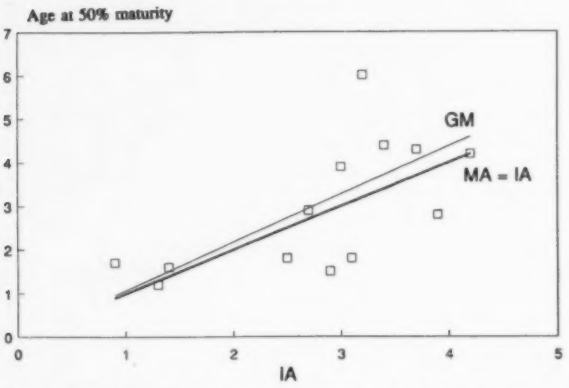

FIG. 4. Plot of age of 50% maturity (MA) against age at maximum growth rate in weight as *dW/dt* (IA) for male white sucker.

LMS-based reweighted GM-estimated regression equations for females and males, respectively, were

$$\log(L_\infty) = 1.70(0.011) - 0.11(0.015)\log(\text{MA}), \quad r = -0.92,\ P = 0.000$$

$$\log(L_\infty) = 1.69(0.031) - 0.16(0.047)\log(\text{MA}), \quad r = -0.75,\ P = 0.001.$$

Growth parameter ω was related negatively to population density for both female and male white sucker, with no populations being identified as outliers (Fig. 6). The GM-estimated regression equations for females and males, respectively, were

$$\log(\omega) = 1.66(0.105) - 0.19(0.065)\log(\text{white sucker population density}), \quad r = -0.72,\ P = 0.018$$

$$\log(\omega) = 1.69(0.104) - 0.19(0.064)\log(\text{white sucker population density}), \quad r = -0.72,\ P = 0.018.$$

MA was considered significantly with population density for both female and male white sucker (Fig. 7). There were no outlier populations in the LMS analysis. The GM regression equations were estimated for females and males, respectively, as

$$\text{MA} = 1.00(0.730) - 0.06(0.010)(\text{white sucker population density}), \quad r = 0.75,\ P = 0.013$$

$$\text{MA} = 1.02(0.484) + 0.04(0.007)(\text{white sucker population density}), \quad r = 0.78,\ P = 0.008.$$

No significant relationships were found between MA of both female and male white sucker versus lake pH, lake area, and maximum depth and the density of ephemeropteran larvae (Table 5). The significant and negative relationships were observed between log(ML) and lake pH for both female and male white sucker, and no outliers were identified (Table 5). The GM-estimated regression equation was also significant between log(ML) of male white sucker and log(lake area) (Table 5). Although the GM-estimated regression equation for females was not significant, the LMS-based reweighted GM regression equation was significant between log(ML) versus log(lake area) with three populations being identified (i.e., Bentshoe, Buck, and Solitaire, Table 5).

Discussion

Male white sucker typically mature at a younger age and smaller size than females (Scott and Crossman 1973). This observation was confirmed in all 13 populations. The great variation in MA and ML among populations agrees with previous reports (Beamish 1970; Trippel and Harvey 1989, 1991). The practicality of proposing a universal MA and ML for white sucker was discussed by Trippel and Harvey (1991).

Beverton (1963) noted that the length at maturity as a fraction of maximum attainable length (ML/L_∞) should be constant for different populations of the same species. In addition, he suggested that differences in this ratio between populations of the same species might indicate differences in the response of fish to environmental selection on maturation. Large variation was observed among populations (Fig. 1). However, within populations the differences between females and males were small and there was an isometric relationship in the ratio of ML/L_∞. The Gullfeather population had a greater value of ML/L_∞ and an older MA than any other population in this study (Table 4). This may result from the very high density of white sucker in this lake (Trippel and

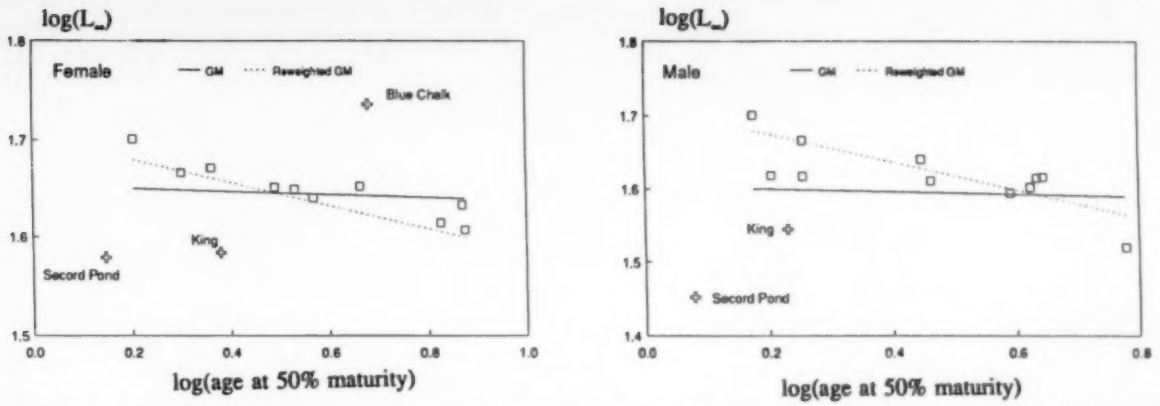

FIG. 5. Geometric means (GM) and least median squares (LMS) based reweighted GM regression analyses between the logarithm von Bertalanffy's estimated maximum attainable length (L_∞) against age at 50% maturity (MA) for female and male white sucker. The populations labelled are the outliers identified in the LMS analysis.

TABLE 5. Summary of regression analyses between maturation parameters and environmental variables for female and male white sucker. log = $\log_{10}$. *Regression line significant at $P = 0.05$.

Variable		GM					Least median squares based reweighted GM					
Y	*X*	b_0	b_1	r^2	*N*	*P*	b_0	b_1	r^2	*N*	*P*	Outlier
					(a) Females							
log(MA)	log(area)	−1.76	1.38	0.11	13	0.26	−1.76	1.38	0.11	13	0.26	None
log(MA)	log(depth[a])	3.50	−2.40	0.01	13	0.93	3.69	−2.45	0.12	12	0.28	Secord
log(MA)	Lake pH	4.43	−0.65	0.22	13	0.10	4.43	−0.65	0.22	13	0.10	None
log(MA)	log(chiro[b])	4.67	−2.09	0.08	11	0.39	2.74	−1.09	0.22	10	0.17	Solitaire
log(MA)	log(ephe + 1[c])	−0.09	1.46	0.07	11	0.42	−0.09	1.46	0.07	11	0.42	None
log(ML)	log(area)	0.93	0.31	0.22	13	0.11	1.27	0.12*	0.88	10	0.00	—[d]
log(ML)	log(depth)	3.80	−1.90	0.01	13	0.82	2.12	−0.51	0.15	12	0.24	Solitaire
log(ML)	lake pH	2.41	−0.16*	0.39	13	0.02	2.41	−0.16*	0.39	13	0.02	None
log(ML)	log(chiro)	3.50	−1.03	0.03	11	0.60	1.91	−0.22	0.27	10	0.13	Solitaire
log(ML)	log(ephe + 1)	1.20	0.60	0.03	11	0.64	1.43	−0.10	0.01	10	0.97	Solitaire
					(b) Males							
log(MA)	log(area)	−2.19	1.57	0.07	13	0.38	−2.19	1.57	0.07	13	0.38	None
log(MA)	log(depth[a])	−6.83	5.85	0.02	13	0.63	−6.83	5.85	0.02	13	0.63	None
log(MA)	Lake pH	4.92	−0.75	0.12	13	0.24	4.92	−0.75	0.12	13	0.24	None
log(MA)	log(chiro[b])	4.41	−2.02	0.06	11	0.48	4.41	−2.02	0.06	11	0.48	None
log(MA)	log(ephe + 1[c])	−0.05	1.09	0.09	11	0.38	−0.05	1.09	0.09	11	0.38	None
log(ML)	log(area)	1.00	0.24*	0.35	13	0.03	1.20	0.17*	0.61	12	0.00	Solitaire
log(ML)	log(depth)	−1.14	2.05	0.02	13	0.62	2.60	−0.93	0.03	12	0.63	Solitaire
log(ML)	lake pH	2.36	−0.16*	0.36	13	0.03	2.36	−0.16*	0.36	13	0.03	None
log(ML)	log(chiro)	3.38	−1.00	0.01	11	0.84	3.38	−1.00	0.01	11	0.84	None
log(ML)	log(ephe + 1)	1.35	0.01	0.01	11	0.76	1.35	0.01	0.01	11	0.76	None

[a]Lake maximum depth (m).
[b]Chironomid density (no./m^2).
[c]Ephemeropteran density (no./m^2).
[d]Three lakes were identified as outliers in the LMS analysis: Bentshoe, Buck, and Solitaire.

Harvey 1991). Many of the mature females did not spawn annually (Trippel and Harvey 1990), and hence the stress of maturation was low. In contrast, the Solitaire population had a much lower density than other populations (Table 1), matured at very young ages, and had a much smaller value of ML/L_∞. Secord Pond is a reservoir stocked with rainbow trout, *Oncorhynchus mykiss*. It is drained about every 8 yr. As a result, there may have been selection for fishes that mature at a young age. However, no significant regressions were observed between ML/L_∞ and any abiotic or biotic variables.

Trippel and Harvey (1987) observed that white sucker in deeper lakes tended to have larger maximum attainable sizes (L_∞). They postulated that this relationship might result from thermal stratification of deeper lakes during the summer months and that white sucker, feeding at night on the much

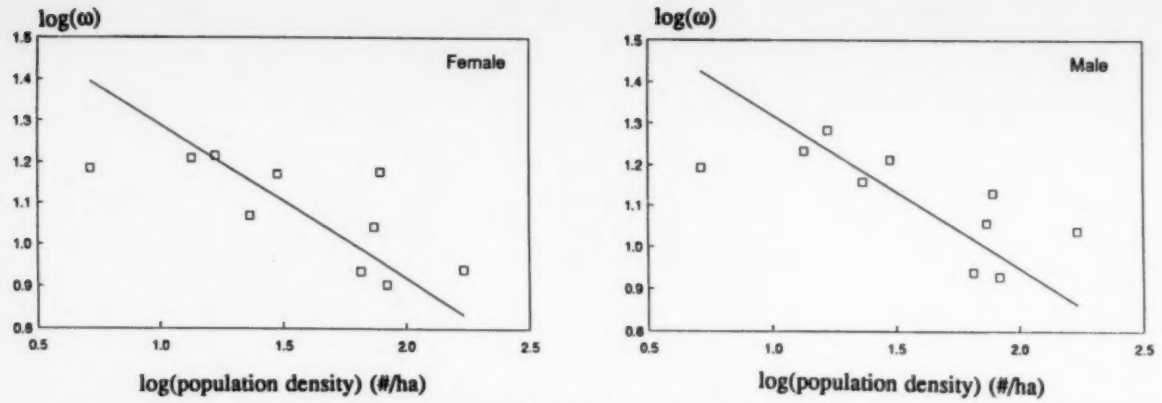

FIG. 6. Plot of the Gallucci and Quinn's ω versus population density calculated as the estimated population size divided by lake area (P/S) for female and male white sucker.

more abundant benthos of the littoral zones, grew faster and attained larger maximum size by reducing their metabolic rate during daylight by residing in cooler deeper water. However, in contrast with maximum attainable size, white sucker maturation parameters tended to be independent of lake maximum depth.

Parameter t_0 in VBGF is the hypothetical age at which length is 0. Because a fish has length at hatching, t_0 tends to be a negative value (Moreau 1987). This has been described as a criterion to judge whether or not VBGF's fit growth data well. Growth parameter ω is, theoretically, the growth rate as dL/dt at t_0, and hence should be related to the growth rate of fish at very young ages (Kingsley 1980; Moreau 1987). Within a fish population, it is well known that individual fish growing rapidly prior to maturity tend to mature earlier than those growing slowly (e.g., Brett 1979; Boyce 1988). The negative and significant regression equation between log-transformed MA and ω in this study (Fig. 2) implies that in a population, individual white sucker with rapid growth rates while young tend to mature at younger ages than those in populations having slower rates of early growth. Thus, hypothesis I was supported by this study. For female white sucker from Secord Pond and McQuaby Lake, the MA's were much lower and higher, respectively, than for other lakes, resulting in these being identified as outliers (Fig. 2). For the Secord Pond population, this may result from the artificial selection for early maturing females. In the McQuaby Lake population, white sucker grow exceptionally rapidly and thus have much larger sizes-at-age relative to white suckers from other populations, but beyond this the reasons for it being an outlier are unclear. The relationship between ω and MA may be driven by the food limitation or spawning site limitation (Trippel and Harvey 1987, 1990). Although significant relationships were not observed between early growth rate ω and chironomid density, a negative and significant relationship was observed between log(population size of white suckers) and log(chironomid density). The GM-estimated equation was log(chironomid density) = 5.22(0.269) − 0.90(0.155)log(population density of white sucker), $r^2 = 0.52$, $P = 0.018$. This suggests that food limitation drove the relationship between early growth rate ω and MA.

Growth of every organism is limited by energy. Energy once acquired and transformed can be stored or it can be used for current maintenance, growth, or reproduction (ultimately the stored energy is used for these purposes or is lost upon the death of the organism). Prior to maturation, fishes mainly use their energy in growth and maintenance. In this life period, fishes usually grow rapidly. As fishes approach maturity, resources are diverted to growth of reproductive tissues, and somatic growth is slowed (Hile 1936; Woodhead 1960; Nikolskii 1965; Shulman 1972; Craig 1987). IA is the age at maximum dW/dt. Thus, based on the trade-off theory between growth and maturation, an hypothesis (II) can be stated as follows: IA has an interdependent relationship with MA. Jensen (1985) found that IA was close to MA for lake whitefish. In this study, a significant regression was found only for male white sucker (Fig. 4). The slope and intercept of this regression equation did not differ significantly from 1 and 0, respectively. This indicates that MA and IA are statistically the same for male white sucker, supporting hypothesis II. However, no such significant relationship existed for female white sucker (Fig. 3, left panel). It is apparent that the MA − IA relationship of females has larger variability than that of males, an indication that female white sucker have larger variations in the relationship between maturation and growth than males. The great variation in the MA − IA relationship for female white sucker was related to lake chironomid abundance. In lakes with abundant chironomid larvae, female white sucker tended to attain IA relatively later than MA (i.e., they mature first, Fig. 3, right panel). Thus with good supplies of chironomid prey, female white sucker tended to mature before attaining their maximum growth rate in weight. The importance of chironomid abundance on growth and maturation of female white sucker may result from the fact that chironomids are perhaps the best nutrient resources (best size and most abundant) available for young white sucker; larger white sucker may prefer larger but often less abundant prey items. The sexual dichotomy in the relationship between (MA − IA) versus chironomid density may be the result of females diverting more resources into gonads and maturing 1–2 yr later.

It has been observed for many species that individuals that mature earlier tend to have smaller maximum attainable length (Brett 1979). However, no significant regression equations were observed between L_∞ and MA using the GM method in this study for both female and male white

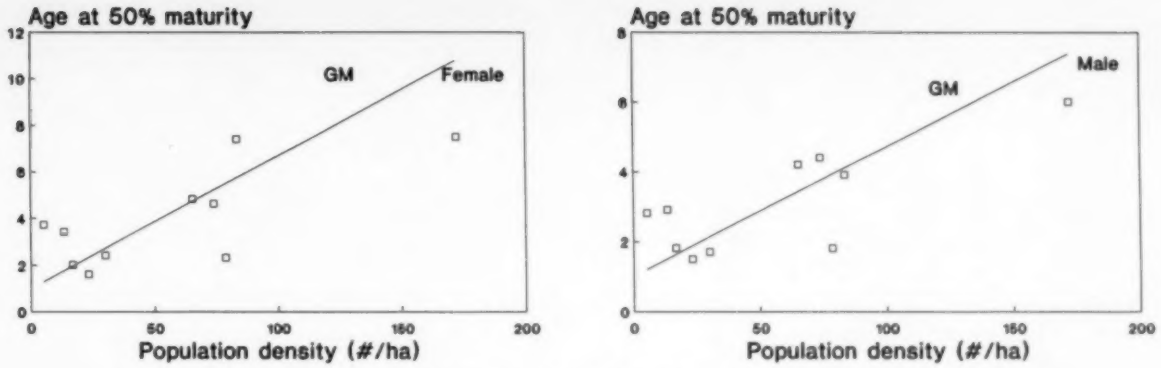

FIG. 7. Plot of age at 50% maturity (MA) versus population density for female and male white sucker.

sucker. When the LMS-based reweighted GM approach was applied, it was observed that fish that matured earlier tended to have larger L_∞ (Fig. 5). Secord Pond and King Lake were identified as outlier populations for both females and males (Fig. 5). Both populations had much smaller L_∞ relative to MA than other populations. These lakes have much smaller maximum depths (6 m) versus the other study lakes, and L_∞ of white sucker is strongly affected by maximum depth (Trippel and Harvey 1987). The outlier of the female white sucker from Blue Chalk Lake may result from the large error variance associated with the estimated L_∞ (Table 4). The reason for having a negative relationship between L_∞ and MA is unclear, and more white sucker populations should be included in a test of hypothesis III.

For a given fish species, age and length at maturity usually vary among populations/stocks (Brett 1979). This has been found to be the response of fishes to changing abiotic and biotic environments, such as food supply, water chemistry, lake morphometry, and population density (e.g., Regier et al. 1969; Wolfert 1969; Winterton 1975; Spangler et al. 1977; Colby and Nepszy 1981). Both female and male white sucker from high-density populations tended to mature at older ages (Fig. 7). This may result from the significant correlation between early growth rate ω versus population density (Fig. 6) and between population density and chironomid density. Thus, competition for food may be involved in the relationships between MA and population density (Trippel and Harvey 1987, 1990). ML's of white sucker tended to be related significantly and positively to the lake area, but such a relationship did not exist for MA (Table 5). This may be due to the lake-size effect on fish sizes at age: a population inhabiting a larger lake usually contains larger sized individuals. ML's of both female and male white sucker also were observed to relate significantly and negatively to lake pH, indicating that white sucker inhabiting a more acid lake tended to mature at a larger size. Trippel and Harvey (1987) reported that white sucker from acid lakes tended to have greater L_∞'s. They attributed this to reduced inter- and intraspecies competition for food as population sizes declined due to recruitment failures and high natural mortality of fishes associated with lake acidification. This may be the reason behind the observed relationship between ML and pH. However, MA seemed not to be affected by lake pH. No other significant relationships were observed between white sucker maturation parameters and the other measured environmental variables. Thus, hypothesis IV was accepted regarding the environmental variables white sucker population density, chironomid density (see the relationship between MA − IA versus chironomid density for female white suckers), lake pH, and lake area.

In conclusion, this study indicates that maturation of white sucker mainly relates to their early growth rate in length, the faster white sucker grow in their young ages, the earlier they attain maturity. This relationship may be driven by food limitation, here measured as chironomid density. Among the measured variables, white sucker population density, chironomid density, lake pH, and area were all associated with white sucker maturation.

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Feeding Ecology of Walleye, *Stizostedion vitreum*, Larvae: Effects of Body Size, Zooplankton Abundance, and Zooplankton Community Composition

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Johnston, T.A., and J.A. Mathias. 1994. Feeding ecology of walleye, *Stizostedion vitreum*, larvae: effects of body size, zooplankton abundance, and zooplankton community composition. Can. J. Fish. Aquat. Sci. 51: 2077–2089.

We conducted laboratory experiments to examine the functional response and conducted comparative laboratory and field studies to examine the prey selection of walleye, *Stizostedion vitreum*, larvae. Maximum consumption rate (C_{max}, micrograms per hour) increased exponentially with walleye mean length whereas the prey abundance at which 90% of C_{max} is attained (D_{90}, prey per litre) increased initially and then declined with mean length. Prey size had no significant effect on C_{max} whereas D_{90} declined significantly with increasing prey size. Selection for relatively larger prey and selection for large cladocerans relative to cyclopoid copepods increased significantly with walleye mean length. Walleye larvae consistently selected most strongly for relatively uncommon prey. Selection for relatively smaller prey and selection for cyclopoid copepods relative to large cladocerans increased significantly with total prey abundance in field studies and some laboratory studies. Variance in prey selection showed a significant, negative relationship with prey abundance in laboratory studies. Selection for medium (≥0.3–0.6 mm body width) relative to small (<0.3 mm) prey declined significantly with increasing temperature in field studies. Our results suggest that the feeding success of walleye larvae may be limited by both the abundance and size composition of the zooplankton communities in natural environments.

Nous avons effectué des expériences en laboratoire pour examiner la réponse fonctionnelle, et des études comparatives en laboratoire et sur le terrain pour déterminer quelles proies choisissent les larves de doré, *Stizostedion vitreum*. Le taux de consommation maximal (C_{max}, microgrammes par heure) augmentait de façon exponentielle avec la longueur moyenne du doré, alors que l'abondance des proies à laquelle 90 % de la C_{max} était obtenue (D_{90}, proies par litre) augmentait au début, puis diminuait avec la longueur moyenne. La taille des proies n'avait pas d'effet significatif sur la C_{max}, alors que la D_{90} diminuait de façon significative avec la taille des proies. Le choix d'une proie relativement plus grosse et le choix de gros cladocères au lieu de copépodes cyclopidés augmentait de façon significative avec la longueur moyenne du doré. Les larves de doré choisissaient de façon très significative des proies relativement peu abondantes. Le choix de proies relativement plus petites et le choix de copépodes cyclopidés au lieu de gros cladocères augmentait de façon significative avec l'abondance de l'ensemble des proies dans les études sur le terrain et dans certaines études en laboratoire. On a observé une corrélation négative significative entre la variance du choix des proies et l'abondance des proies dans les études en laboratoire. Le choix de proies de taille moyenne (≥0,3–0,6 mm) au lieu de proies de petite taille (<0,3 mm) diminuait de façon significative avec la température dans les études sur le terrain. Nos résultats indiquent que l'alimentation des larves de doré peut être limitée par l'abondance et la taille des individus des communautés de zooplancton présentes dans les milieux naturels.

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The larval period is considered critical in the life history of many fish species (Blaxter 1988) and starvation and predation are believed to be the major causes of larval mortality (Hunter 1981). Successful feeding during the larval period reduces the risk of starvation and ensures rapid growth through the size-classes most vulnerable to predation. Studies of the feeding ecology of fish larvae are thus essential for understanding the factors influencing the feeding success, and ultimately the survival, of fish larvae.

Feeding in fish may be affected by both the quantity and quality of available prey. Fish, like most animals, demonstrate a functional response to prey abundance (Ivlev 1961). The rate of prey consumption increases with increasing prey abundance, with the most rapid change occurring at low prey abundances (Holling 1959). Among the qualitative characteristics of prey, size is one of the most important.

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TABLE 1. Summary of laboratory feeding experiments conducted in 1988. Walleye larvae were obtained from Crean Lake, Saskatchewan, and from the Swan Creek stock of Lake Manitoba. Each experimental run involved 21 experimental aquaria (see text and Table 2). Experimental runs were replicated (blocked) two or three times on each experimental date. Feeding time represents a mean calculated for all experimental aquaria. Dry weights for individual larvae were estimated from length – dry weight relationships from previous studies (T.A. Johnston, unpublished data).

		Walleye size ($\bar{X}$ ± 1 SD)				
Date	Walleye stock	Length (mm)	Dry weight (mg)	Replicates	Larvae·aquarium^{-1}	Feeding time (min)
27 May	Crean Lake	9.45 ± 0.06	0.50 ± 0.02	2	15	81
28 May	Crean Lake	9.67 ± 0.08	0.55 ± 0.02	3	18	65
19 May	Lake Manitoba	10.1 ± 0.15	0.71 ± 0.05	2	12	67
31 May	Crean Lake	10.9 ± 0.28	0.95 ± 0.11	3	12	55
22 May	Lake Manitoba	11.2 ± 0.19	1.13 ± 0.12	3	10	69
29 May	Lake Manitoba	13.9 ± 0.47	2.88 ± 0.42	3	6	44
6 June	Crean Lake	14.8 ± 0.93	3.64 ± 0.90	2	4	18

The size of a prey item often determines how easily it can be detected, captured, and ingested. Size may also be an index of the nutritive value of a prey item. In addition, both the quantitative and qualitative effects of prey on feeding will vary with the development of the fish. The ecology of fishes changes during ontogeny (Werner and Gilliam 1984) and thus, prey assemblages that are optimal for feeding by young larvae may not be optimal for older larvae and vice versa.

Li and Mathias (1982) noted a critical period of high mortality at the onset of exogenous feeding in walleye, *Stizostedion vitreum*, larvae and hypothesized that food limitation during this period may be the major cause of larval mortality. However, since the early work of Mathias and Li (1982), few detailed studies on the feeding ecology of walleye larvae have been carried out. No studies, to our knowledge, have examined the effects of prey size on the functional response or how the functional response changes during larval development. Although several studies have examined ontogenetic changes in prey selection of walleye larvae (Raisanen and Applegate 1983; Graham and Sprules 1992), none has examined the influence of prey abundance or prey community composition on prey selection.

The purpose of this study was to conduct a detailed examination of the factors influencing the feeding ecology of walleye larvae. Our objectives were (i) to examine the effects of walleye body size and prey size on the functional response of walleye larvae and (ii) to examine the effects of walleye body size, prey community composition, and prey abundance on prey selection by walleye larvae. The results of these studies are interpreted in relation to the structure of zooplankton communities, and the implications for walleye feeding success in natural waterbodies are discussed.

Materials and Methods

Experimental Procedure

This research was carried out at the Department of Fisheries and Oceans' Dauphin Lake Walleye Rehabilitation and Research Station at Methley Beach (Dauphin Lake), Manitoba. Laboratory experiments were conducted in May and June of 1988. Field data were collected in May and June of 1987, 1988, and 1989 from culture ponds at the field station. Details of the pond environments and their management are provided in Johnston et al. (1992).

Hatchery-raised walleye larvae from two stocks (Table 1) were brought into the laboratory within 1–3 d after hatching and raised in 120-L glass stock aquaria at 20°C. The sides of the stock aquaria were covered with translucent green plastic sheeting. Wild zooplankton swept from the field station culture ponds were provided at least 1 d prior to the onset of feeding and maintained at densities >200·L^{-1} thereafter. Experimental units were 13-L rectangular glass aquaria covered in green plastic sheeting and filled with 10 L of 20°C (range 19–21°C), filtered (through 45-μm Nitex® mesh) pond water. Light levels over the aquaria ranged from 0.6 to 0.9 μE·m^{-2}·s^{-1}.

Laboratory experiments were conducted on seven dates corresponding to walleye mean lengths of 9.45–14.8 mm (Table 1). Twenty-one aquaria were used in each experimental run and two or three runs were performed in a given day (Table 1). Within a run, each aquarium was randomly assigned a different zooplankton treatment and the runs represented replicates blocked for the time of day. Prior to each run, larvae were removed from the stock aquaria, randomly assigned to the experimental aquaria, and left in total darkness for 4 h to allow their guts to clear. The number of larvae added to each experimental aquarium ranged from 18 for experiments with small larvae to 4 for experiments with the largest larvae (Table 1). Zooplankton treatments were added to the aquaria during the preexperimental period. Zooplankton were swept from the Methley culture ponds and passed through a series of Nitex® sieves to separate them into three size fractions hereafter referred to as S (small, 183–300 μm), M (medium, 300–509 μm), and L (large, 509–1050 μm). Samples of each fraction were preserved in 5% buffered formalin for each experimental run to determine size and species composition. Zooplankton additions to the experimental aquaria are summarized in Table 2. One aquarium received no zooplankton. Twelve aquaria were randomly assigned one of the three size fractions at one of four abundances (15, 30, 60, or 120 prey·L^{-1}). The eight remaining aquaria were given assemblages of these three fractions in one of two ratios, A_{EQ} (equal proportions, S:M:L = 1:1:1) or A_{UNEQ} (unequal proportions, S:M:L = 9:3:1), at one of four abundances (40, 60, 100, or 180 prey·L^{-1}). We commenced experiments by turning on the lights and terminated experiments by removing the fish, sacrificing them in MS-222 (*m*-aminobenzoate methanesulfonate), and preserving them in 5% buffered formalin. The larvae were allowed to feed for a long enough duration to ingest a minimum of 10 prey per aquarium but for a short enough duration to prevent satiation. Total feeding time

TABLE 2. Experimental design for laboratory feeding experiments. Zooplankton treatments of various size compositions (C = control (no addition), S = small, M = medium, L = large, A_{EQ} = equal assemblage, A_{UNEQ} = unequal assemblage (see text)) were added in varying abundances to 21 experimental aquaria as indicated. This design was replicated in two or three blocks on each experimental date (see Table 1).

	Zooplankton treatment					
Abundance (prey·L^{-1})	C	S	M	L	A_{EQ}	A_{UNEQ}
0	√					
15		√	√	√		
30		√	√	√		
40					√	√
60		√	√	√	√	√
100					√	√
120		√	√	√		
180					√	√

ranged from 81 min for fist-feeding larvae to 18 min for the largest larvae (Table 1).

Preserved larvae were measured to the nearest 0.1 mm using an ocular micrometer for small larvae and a scientific ruler for larger larvae. Length was measured as total length until a definite fork appeared in the tail and as fork length thereafter. Preserved lengths were converted to fresh lengths using the formula of Johnston and Mathias (1993). Gut contents were identified to genus, enumerated, and measured to the nearest 0.02 mm with an ocular micrometer. Zooplankter length was measured from the anteriormost point of the body to the base of the tail spine in Cladocera and to the end of the caudal rami in Copepoda. Zooplankter width was measured as the second longest body dimension. This corresponded to body width in Copepoda and to dorsoventral depth in Cladocera. Zooplankter dry weight was estimated using length – dry weight formulae developed for zooplankton of the Methley culture ponds (Johnston 1995). Food consumption rate was calculated for each fish as the dry weight of stomach contents (micrograms) divided by the total foraging time (hours).

Samples of walleye larvae were trawled or seined from each of the four Methley culture ponds between 09:00 and 20:00 at roughly 4-d intervals following stocking. Subsamples of ~20 fish (range 15–38) from each trawl or seining were processed and analyzed in the same manner as the laboratory experimental fish. Quantitative zooplankton samples were collected within 24 h of the fish samples using oblique tows (1987) or vertical tows (1988 and 1989) with Wisconsin nets (25-cm diameter; 73-μm mesh). Mean zooplankton abundances and species and size distributions were calculated from these samples. Detailed descriptions of the zooplankton and larval fish sampling procedures and the pond zooplankton community are given in Johnston et al. (1992).

Statistical analyses were divided into two parts. The first examined the functional response of walleye larvae using only laboratory data. The second examined prey selection of walleye larvae using both laboratory and field data.

Functional Response Analysis

The functional response was analyzed separately for each of the seven experimental dates (Table 1). The percentage of fish feeding increased asymptotically with zooplankton abundance and the asymptote was below 100% for all but the largest fish. This suggested that some nonfeeders were limited by the zooplankton treatment whereas others would not have fed regardless of the zooplankton treatment. It was this latter group of fish that we wished to eliminate from further analyses. However, because it was impossible to determine why each individual nonfeeder did not feed, we decided to randomly eliminate a portion of nonfeeders equal to (total number of experimental fish) × (1 − asymptotic percent feeding) from each experimental run.

Scatter plots of consumption rate versus zooplankton abundance indicated that a Holling type II functional response was the most appropriate model for most zooplankton treatments and fish sizes. We used a reparameterized version of the Holling disc equation (Holling 1959) of the form

$$(1)\quad C = 9pC_{max}/(D_{90} + 9p)$$

where C is consumption rate (micrograms per hour), p is prey abundance (prey per litre), and C_{max} and D_{90} are fitted parameters representing maximum consumption rate and the prey abundance at which 90% of the maximum consumption rate is attained, respectively. These model parameters can be expressed in terms of the original model parameters as

$$(2)\quad C_{max} = 1/f$$

and

$$(3)\quad D_{90} = 9/af$$

where f is handling time (hours per microgram) and a is the attack coefficient (micrograms per litre per hour). This model assumes that prey abundance remains constant or declines negligibly during the experiment, an assumption frequently violated in experiments with the largest larvae. We accounted for this problem by using the geometric mean of initial and final prey abundances rather than initial prey abundance as the independent variable. Data from experimental aquaria in which prey depletion was severe (>80%) were eliminated from further analyses. Mean consumption rate was calculated for each experimental aquarium and the functional response model was fit to the aquarium means using nonlinear least-squares (NLIN procedure, SAS® Institute Inc. 1985) and the method of iterative reweighting (Holland and Welsch 1977). Consumption rate means were weighted by the inverse of their predicted standard errors as estimated from the empirical variance–mean relationship (Taylor power plot, Elliott 1977). For each experimental date, the functional

TABLE 3. Comparison of environmental and experimental conditions of laboratory and field studies examining prey selection by walleye larvae. Field studies were carried out in the Methley Beach walleye culture ponds. Prey size and prey species categories are defined in the text.

Variable	Range of observed conditions	
	Laboratory	Field
Walleye		
Mean length (mm)	9.45–14.8	8.90–26.1
Feeding state	Guts rarely full	Guts usually full
Environment		
Temperature (°C)	19–21	12.9–24.3
Light intensity ($\mu E \cdot m^{-2} \cdot s^{-1}$)	0.6–0.9	100–1000[a]
Prey abundance ($prey \cdot L^{-1}$)	15–180	14–388
Prey size relative abundances (%)		
Small	1–76	15–96
Medium	5–94	3–78
Large	1–89	1–55
Prey species relative abundances (%)		
Cyclopoids	4–96	12–93
Large Cladocera	2–90	3–82
Others	1–13	1–42

[a]Measured between the surface and a depth of 150 cm at noon on an overcast day when pond Secchi depth was >200 cm.

response model was fit to each of the individual zooplankton treatments (S, M, L, A_{EQ}, and A_{UNEQ}). The NLIN procedure provided estimates for the means and asymptotic standard errors of C_{max} and D_{90}. Changes in the functional response with respect to walleye size and prey size were examined by conducting univariate tests on the model parameters.

We tested the hypothesis that C_{max} varied with respect to walleye size by regressing $\log_e(C_{max})$ against $\log_e$(mean length) (GLM procedure, SAS® Institute Inc. 1985). Because the relationship between D_{90} and walleye mean length appeared complex, we tested the hypothesis that D_{90} varied with respect to experimental date using ANOVA (GLM procedure, SAS® Institute Inc. 1985) with experimental date treated as a class variable. In this latter analysis, variation in D_{90} due to experimental date included the effects of walleye mean length and also included variation due to other effects such as fish stock. Parameter estimates from all zooplankton treatments (S, M, L, A_{EQ}, and A_{UNEQ}) were used in both analyses and D_{90} estimates were weighted by the inverse of their standard errors in the latter analysis. Tests of significance were based on the partial (type III) sums of squares to account for the effect of zooplankton treatment.

We tested the hypothesis that C_{max} varied with respect to prey size using ANCOVA (Freund and Littell 1981) where walleye length was treated as a covariate. To test the hypothesis that D_{90} varied with respect to prey size, we conducted an ANOVA (GLM procedure, SAS® Institute Inc, 1985) with experimental date treated as a block effect. In these analyses, we compared parameter estimates from only the S, M, and L zooplankton treatments. Our analysis of the effects of prey size on D_{90} required the further assumption that there was no interaction between the effects of experimental date and prey size on D_{90}.

Prey Selection Analysis

We examined prey selection using both laboratory and field data. From the laboratory experiments, we used data from all zooplankton treatments, but primarily from the assemblage treatments (A_{EQ} and A_{UNEQ}). Ambient experimental conditions differed between laboratory and field studies (Table 3). Laboratory experiments provided more even distributions in the range of prey abundances and relative prey abundances whereas field data covered a wider range of fish sizes and water temperatures.

Prey selection was measured using an electivity index, ε proposed by Chesson (1983). Electivity for the ith prey category, ε_i, was calculated from the formulae

$$(4)\quad \varepsilon_i = (m\alpha_i - 1)/((m - 2)\alpha_i + 1)$$

and

$$(5)\quad \alpha_i = (r_i/n_i)/\Sigma(r_i/n_i)$$

where α_i is Chesson's (Manly's) preference index, r_i is the proportion of consumed prey that was of category i, n_i is the proportion of available prey that was of category i, and m is the number of prey categories available. ε_i can take values of -1 to 1, with 0 representing neutral selection. The above formula for α_i assumes that the prey community composition changes negligibly during the experiment. For this reason, we eliminated all laboratory data from aquaria in which >10% of available particles were consumed during the course of an experiment. Prey community composition was assumed constant within each sampling period for all field data. Electivity indices may sometimes be strongly influenced by prey items that are rare in the environment (Lechowicz 1982; Confer and Moore 1987). To reduce this possibility, were eliminated all data where $n_i < 0.005$ in field studies or $n_i < 0.01$ in laboratory studies. A slightly more conservative criterion was used for the laboratory studies because of the greater risk of prey depletion in small volumes.

Electivity indices were calculated using both prey size and prey species categories. In both cases, food items were divided into three categories. To examine size selection, we

TABLE 4. First (PC1) and second (PC2) principal components calculated for vectors of electivity indices (ε_i). Values in parentheses represent the percentage of total variation in electivity accounted for by each principal component. Interpretations of the principal components in terms of the prey categories of the original indices were made in relation to the magnitude and sign of the coefficients. Prey size categories corresponding to ε_1, ε_2, and ε_3 were small (<0.3 mm body width), medium (≥0.3–0.6 mm), and large (≥0.6 mm) zooplankton, respectively. Prey species categories corresponding to ε_1, ε_2, and ε_3 were cyclopoid copepods (mostly (*Cyclops bicuspidatus*), large cladocerans (*Daphnia pulex*, *Ceriodaphnia quadrangula*, *Simocephalus vetulus*), and other zooplankton (*Bosmina longirostris*, *Chydorus sphaericus*, *Diaptomus siciloides*), respectively.

Electivity index vector	Principal component formulae	Interpretation
Laboratory studies		
Prey size	PC1 = $-0.77\cdot\varepsilon_1 + 0.53\cdot\varepsilon_2 + 0.36\cdot\varepsilon_3$ (74.2%)	Medium and large relative to small
	PC2 = $-0.07\cdot\varepsilon_1 + 0.62\cdot\varepsilon_2 + 0.78\cdot\varepsilon_3$ (22.8%)	Large relative to medium
Prey species	PC1 = $-0.76\cdot\varepsilon_1 + 0.47\cdot\varepsilon_2 + 0.44\cdot\varepsilon_3$ (58.8%)	Large cladocerans and others relative to cyclopoids
	PC2 = $0.06\cdot\varepsilon_1 - 0.63\cdot\varepsilon_2 + 0.78\cdot\varepsilon_3$ (38.7%)	Others relative to large cladocerans
Field studies		
Prey size	PC1 = $0.30\cdot\varepsilon_1 + 0.54\cdot\varepsilon_2 - 0.78\cdot\varepsilon_3$ (78.7%)	Medium and small relative to large
	PC2 = $-0.76\cdot\varepsilon_1 + 0.63\cdot\varepsilon_2 + 0.15\cdot\varepsilon_3$ (19.4%)	Medium relative to small
Prey species	PC1 = $0.56\cdot\varepsilon_1 - 0.70\cdot\varepsilon_2 + 0.45\cdot\varepsilon_3$ (62.9%)	Cyclopoids and others relative to large cladocerans
	PC2 = $-0.67\cdot\varepsilon_1 - 0.05\cdot\varepsilon_2 + 0.74\cdot\varepsilon_3$ (35.4%)	Others relative to cyclopoids

classified prey by maximum body width: <0.3, ≥0.3–0.6, and ≥0.6 mm. This classification roughly corresponded to the zooplankton size treatments S, M, and L, respectively. We used prey width rather than length, as it is a more consistent selection criterion across a wide range of prey types for gape-limited fishes (Hunter 1981; Hambright 1991). To examine species selection, zooplankton were categorized as cyclopoids (mostly *Cyclops bicuspidatus*), large cladocerans (*Daphnia pulex*, *Ceriodaphnia quadrangula*, *Simocephalus vetulus*), and others (*Bosmina longirostris*, *Chydorus sphaericus*, *Diaptomus siciloides*). Means and standard errors of ε were estimated for each experimental aquarium in laboratory studies and for each pond and sampling date in field studies by jackknifing (Krebs 1989) using the FORTRAN subroutine of Matloff (1980).

We tested the hypotheses that prey selection by walleye larvae varied with respect to walleye size, relative prey abundance, total prey abundance, and water temperature using regression analysis (SAS® GLM procedure, Freund and Littell 1981). Because the electivity indices were highly correlated, we chose to use the principal components of the electivity index vectors as dependent variables rather than the electivity indices themselves. This allowed us to examine most of the variability in prey electivity without repeating identical univariate analyses on highly correlated dependent variables. Principal components for the prey size and prey species electivity index vectors for both laboratory and field data (four vectors in total) were calculated from the variance–covariance matrices using the SAS® PRINCOMP procedure (SAS® Institute Inc. 1985). Generally, PC1 compared one electivity index against the combination of the other two in the vector whereas PC2 compared the latter two indices. Thus, as dependent variables, the principal components represented the electivity for one group of prey relative to another (Table 4).

Eight separate regressions were conducted, one for each of the principal components of Table 4. The independent variables in each regression analysis were $\log_e$(mean length) (LEN), $\log_e$(relative prey abundance) (RA), total prey abundance (DENS, prey per litre), and water temperature (TEMP, degrees Celsius). The variable TEMP was used only in analyses of field data. Relative prey abundance was calculated as the ratio of prey abundances as indicated by the interpretations of the principal components in Table 4. Thus, the calculation of RA was different for each regression. For example, when PC1 of the prey size electivity index for laboratory studies was used as a dependent variable the relative prey abundance was calculated as the abundance of medium and large prey divided by the abundance of small prey (Table 4). The significance of an independent variable's effect on the principal component was determined by examining the partial (type III) sums of squares. We plotted model residuals against the independent variables to examine the variable's influence on prey selection and to determine if transformation or weighting was required (Draper and Smith 1981).

Results

Functional Response

We examined trends in the functional response parameters with respect to walleye size by plotting means of C_{max} and D_{90} (adjusted for zooplankton treatment, LSMEANS option, GLM procedure, SAS® Institute Inc. 1985) for each experimental date against walleye mean length (Fig. 1). Maximum consumption rate, C_{max}, increased curvilinearly with walleye mean length over the size range of 9.45–14.8 mm (Fig. 1a). This relationship was defined by the equation $\log_e(C_{max}) = 16.7 + 8.38\cdot\log_e$(mean length) (ANOVA, $F = 147$, df = 1,27, $P < 0.001$, $R^2 = 0.85$). The prey abundance at which 90% of maximum consumption rate was attained, D_{90}, increased from 45·L^{-1} at first feeding to a high of 387·L^{-1} for 10.1-mm larvae and then declined to 55·L^{-1} for the largest larvae examined (Fig. 1b). D_{90} differed significantly among experimental dates (ANOVA, $F = 3.47$, df = 6,22, $P = 0.015$). Multiple comparisons of the means (TUKEY option, GLM procedure, SAS® Institute Inc. 1985) indicated significant differences ($P < 0.05$) between all means with the exception of two groupings. There were no significant differences in D_{90} among experimental dates corresponding to walleye mean lengths of 10.9, 11.2, and 13.9 mm and between experimental dates corresponding to walleye mean lengths of 9.45 and 14.8 mm.

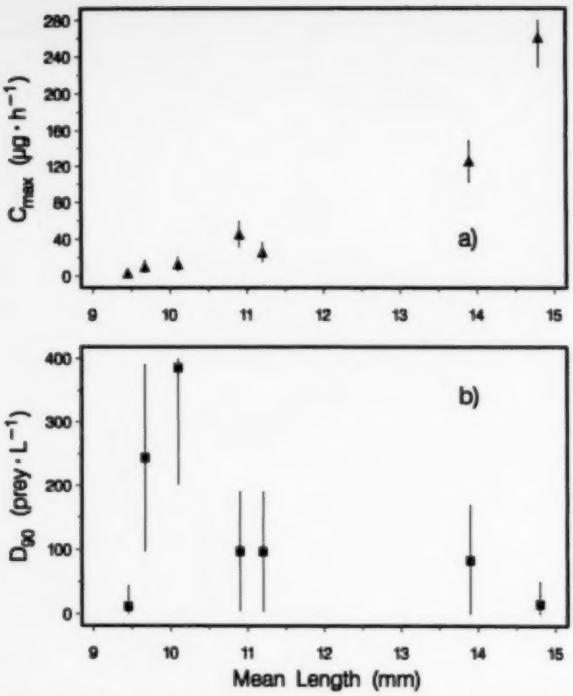

FIG. 1. Relationships between functional response model parameters and walleye mean length for walleye larvae feeding on zooplankton in the laboratory. Parameters are (a) maximum consumption rate (C_{max}) and (b) prey density at which 90% of the maximum consumption rate was attained (D_{90}). The functional response model was a modified version of the Holling disc equation (equation 1, see text). Symbols represent means ± 1 SE. Means were adjusted for zooplankton size treatment.

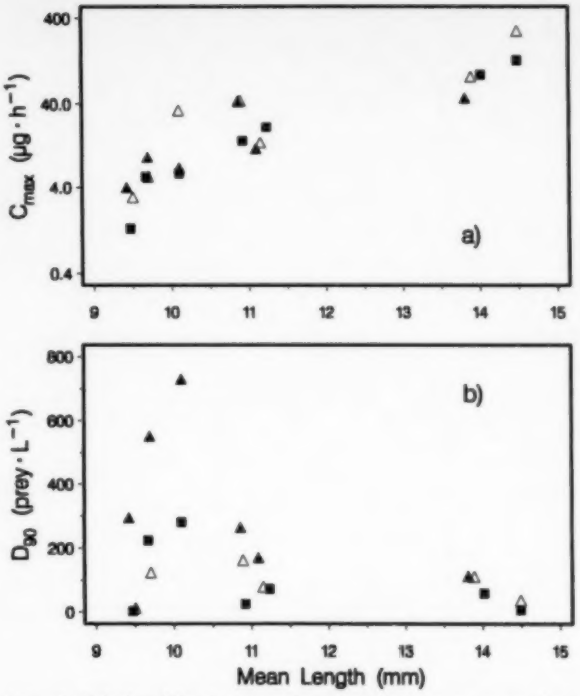

FIG. 2. Relationships between functional response parameters and walleye mean length for walleye larvae feeding on small (183–300 μm, ▲), medium (300–509 μm, △), and large (509–1050 μm, ■) zooplankton in the laboratory. Parameters were (a) maximum consumption rate (C_{max}) and (b) prey abundance at which 90% of the maximum consumption rate was attained (D_{90}). Symbols represent single fitted parameter estimates. The functional response model was a modified version of the Holling disc equation (equation 1, see text). A single observation representing the D_{90} value for 10.1-mm walleye feeding on medium-sized zooplankton (D_{90} = 1951·L^{-1}) is not shown. Note the logarithmic scale in Fig. 2a.

We examined trends in the functional response parameters with respect to prey size by plotting estimates of C_{max} and D_{90} for each of the S, M, and L zooplankton treatments against walleye mean length (Fig. 2). For the smaller (9.45–9.67 mm) larvae, C_{max} was highest when feeding on small prey whereas the the larger (11.2–13.9 mm) larvae, C_{max} was lowest when feeding on small prey (Fig. 2a). However, this interactive effect of zooplankton size and fish size on C_{max} was not statistically significant. Slopes of the $\log_e(C_{max})$ versus $\log_e$(mean length) relationships were not significantly different among zooplankton size treatments (ANCOVA, F = 0.97, df = 2,14, P = 0.40), and following correction for the length covariate, there was no significant difference in C_{max} between zooplankton size treatments (ANCOVA, F = 0.74, df = 1,16, P = 0.49).

The relationship between D_{90} and walleye mean length was qualitatively similar for all zooplankton size treatments, with the highest D_{90} estimates observed in 10.1-mm larvae (Fig. 2b). Generally, D_{90} was highest for small prey and lowest for large prey. The means of D_{90} adjusted for experimental date were 273, 146, and 122·L^{-1} for the S, M, and L treatments, respectively. An unusually high D_{90} value (1951·L^{-1}) was estimated for 10.1-mm larvae feeding on medium-sized zooplankton. We attributed this to unusually high mean consumption rates observed in two experimental aquaria which greatly influenced the fit of the model, and therefore, we excluded this point from further analyses. With this outlier eliminated, there was a significant effect of zooplankton size on D_{90} (ANOVA, F = 5.61, df = 2,10, P = 0.023). Multiple comparisons of the treatment means (TUKEY option, GLM procedure, SAS® Institute Inc. 1985) indicated significant differences among all zooplankton sizes (P < 0.05). Thus, the zooplankton abundance necessary to attain 90% of C_{max} declined significantly with increasing zooplankton size.

These analyses indicated that the shape of the walleye functional response curve varies with prey size and with walleye body size (Fig. 3). Our models predict that 9.67-mm larvae can consume large prey at a faster rate than small prey if prey abundance is low (<40 prey·L^{-1}) and consume small prey at a faster rate than large prey if prey abundance is high (Fig. 3a). Larger larvae (11.2 mm) can consume large prey at a faster rate than small prey over the entire range of prey abundances examined in this study (Fig. 3b). Based on these observations, we hypothesized that if walleye larvae selectively fed on prey sizes that maximized their consumption rate, then selection for larger relative to smaller prey would decline with increasing prey abundance. Furthermore, we predicted that this trend would be most prominent

among first-feeding walleye larvae. We examined these predictions in our analysis of prey selection.

Prey Selection

Prey selection was strongly influenced by walleye size and this was most apparent in the field data (Fig. 4 and 5). Electivity for small prey was positive only for the smallest walleye larvae and declined with increasing walleye length. Medium-sized prey were positively selected by walleye from first-feeding to a mean length of ~20 mm but declined in importance thereafter. Electivity for large prey was negative among first-feeders but increased steadily as the larvae grew. When electivity was calculated based on taxonomic rather than body-size prey categories the trends were less distinct (Fig. 5). The greatest variability in prey species electivity was seen in 8- to 12-mm larvae. In larger larvae, large cladocerans were generally selected over cyclopoids or other zooplankton. However, strong positive electivity for cyclopoids and negative or neutral electivity for large cladocerans by larger larvae (mean length >20 mm) was observed on several occasions. Positive electivity for zooplankton other than cyclopoids or large cladocerans was observed only among the earliest larval stages. The "other" prey species in these cases was primarily *Bosmina longirostris*.

Independent variables representing walleye size, relative prey abundance, total prey abundance, and water temperature accounted for significant amounts of variation in prey electivity by walleye based on our regression analyses using principal components as dependent variables (Table 5). Coefficients of determination (R^2) for the complete regression models (all variables included) ranged from 2 to 19% for the laboratory studies and from 26 to 63% for the field studies. Residuals from some of the complete models (three of four models using PC2 as the dependent variable) had nonnormal distributions that we could not correct by transformation or weighting of the dependent variables. This may have reduced the power of these tests somewhat and they should therefore be considered conservative.

Regression analyses confirmed the strong influence of walleye size on prey electivity (Table 5). The signs of the regression parameters (b_i) for the variable LEN indicated that selection for larger relative to smaller prey was an increasing function of walleye mean length (Table 5). The effect of LEN was significant in six of the eight regression models (Table 5). In laboratory studies, electivity for medium and large prey relative to small prey (prey size, PC1) and electivity for noncyclopoid relative to cyclopoid prey (prey species, PC1) were both positively related to mean length. When differences in the interpretations of principal components were taken into account the trends seen in field data agreed well with the laboratory data (Table 5). For the pond walleye, electivity for medium and small relative to large prey (prey size, PC1) decreased significantly and electivity for medium relative to small prey (prey size, PC2) increased significantly with walleye mean length. Electivity for cyclopoids and others relative to large cladocerans (prey species, PC1) and for others relative to cyclopoids (prey species, PC2) both declined significantly with increasing walleye length. Thus, the regression analyses confirmed the qualitative patterns observed in scatter plots of the original electivity indices (Fig. 4 and 5).

Regression parameters for the variable RA indicated that electivity of one prey group relative to another was consistently

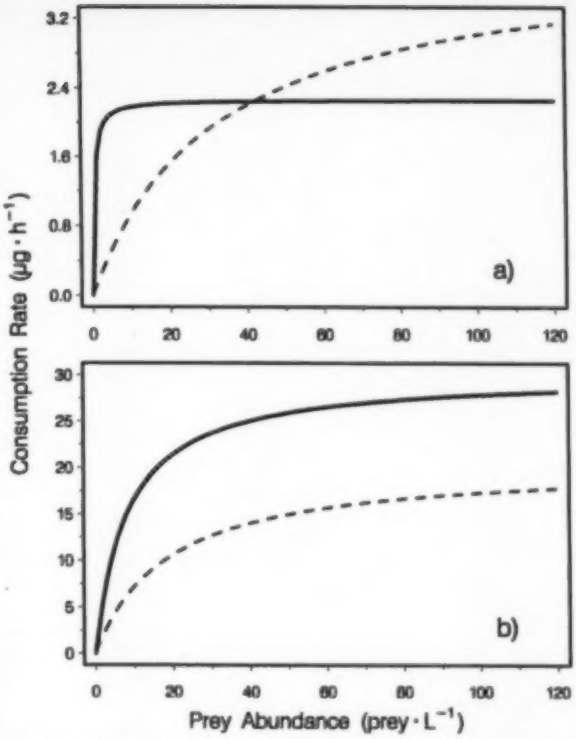

FIG. 3. Predicted functional responses of (a) 9.67-mm mean length and (b) 11.2-mm mean length walleye larvae feeding on small (183–300 μm, ---) or large (509–1050 μm, —) zooplankton. The functional response model was a modified version of the Holling disc equation (equation 1, see text) and parameters were estimated from laboratory feeding experiments. Note the difference in scales of consumption rate axes in Fig. 3a and 3b.

negatively related to the relative abundance of the two groups (Table 5). The effect of RA was significant in four of the eight regression models. In laboratory studies, electivity for medium and large relative to small prey (prey size, PC1) and electivity for noncyclopoids relative to cyclopoids (prey species, PC1) declined significantly with increasing relative prey abundance. In field studies, electivity for medium relative to small prey (prey size, PC2) and electivity for cyclopoids and other zooplankton relative to large cladocerans (prey species, PC1) showed significant, negative relationships with RA.

The effect of prey abundance (DENS) on electivity was negligible in the laboratory studies ($F \leq 0.59$, $P \geq 0.45$, Table 5). However, plots of residuals from the complete model versus DENS indicated that error variance declined with increasing prey abundance. When the variance of the residuals was calculated at DENS intervals of $20 \cdot L^{-1}$ for each of the four principal component models and regressed against prey abundance, a significant, negative relationship was obtained (ANOVA, $F = 8.08$, df = 1,22, $P = 0.0095$). Thus, the variation in prey electivity declined significantly with increasing prey abundance. We reconducted the original regression analyses using a weighting function proportional to the inverse of the predicted residual variance to account for this pattern. However, in the weighted analysis

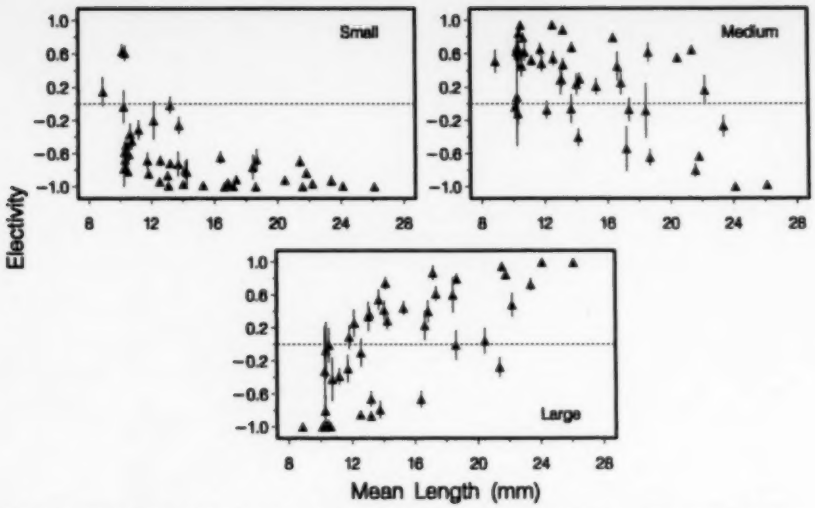

FIG. 4. Scatter plots of electivity, ε, for small (<0.3 mm body width), medium (≥0.3–0.6 mm), and large (≥0.6 mm) prey items by walleye larvae versus walleye mean length. Larvae were sampled from the Methley Beach walleye culture ponds in 1987, 1988, and 1989. Symbols represent jackknifed means ± 1 SE. The broken reference line indicates neutral selection.

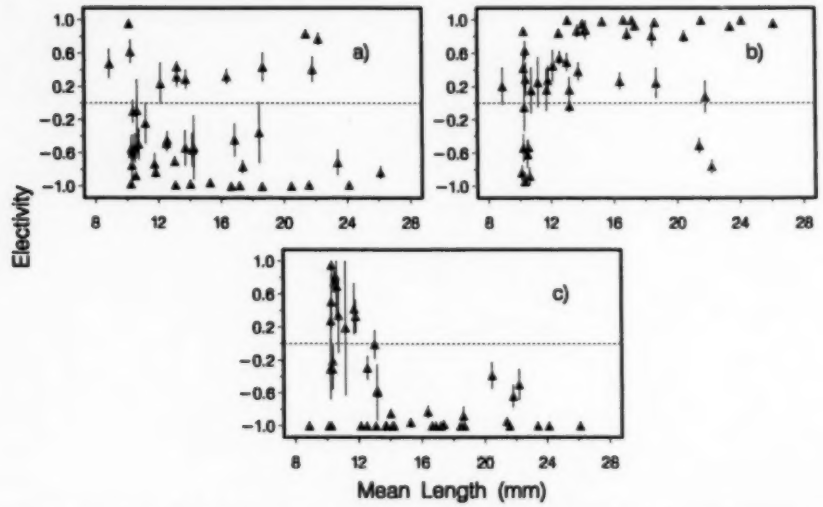

FIG. 5. Scatter plots of electivity, ε, for (a) cyclopoid copepods (mostly *Cyclops bicuspidatus*), (b) large cladocerans (*Daphnia pulex*, *Ceriodaphnia quadrangula*, *Simocephalus vetulus*), and (c) other crustacean zooplankton (*Bosmina longirostris*, *Chydorus sphaericus*, *Diaptomus siciloides*) by walleye larvae versus walleye mean length. Larvae were sampled from the Methley Beach walleye culture ponds in 1987, 1988, and 1989. Symbols represent jackknifed means ± 1 SE. The broken reference line indicates neutral selection.

the effects of DENS on electivity were still not significant ($F \leq 0.99$, $P \geq 0.32$).

Prey abundance had a stronger effect on prey selection under field conditions (Table 5). Electivity for medium and small relative to large prey (prey size, PC1) and electivity for cyclopoids and others relative to large cladocerans (prey species, PC1) were both significantly and positively related to prey abundance. Plots of complete model residuals versus DENS were constructed as for the laboratory studies. The trend of decreasing residual variance with increasing prey abundance was apparent, but not as strong as the trend observed in the laboratory data. When residual variance was calculated as described above and regressed against DENS the relationship was not significant (ANOVA, $F = 0.41$, df = 1,22, $P = 0.53$).

From our functional response analysis, we predicted that when several prey sizes were available in equal proportions, selection for small relative to larger prey would increase with increasing prey abundance and that this trend would be strongest among first-feeding larvae. The observed effect

TABLE 5. Results of regression analyses using principal components (PC1 and PC2) of electivity indices as dependent variables (Y) and $\log_e$(mean walleye length) (LEN), $\log_e$(relative prey abundance) (RA, see text), total prey abundance (DENS, prey·L^{-1}), and water temperature (TEMP, °C), as independent variables (X_i). Principal component formulae and interpretations are listed in Table 4. Parameter estimates are indicated by b_i. F-statistics were claculated based on the partial (type III) sums of squares. Degrees of freedom for the test statistics were 1 and $n - 4$ for the laboratory studies and 1 and $n - 5$ for the field studies, where n is the number of observations (means).

Electivity vector	n	Y	X_i	b_i	F	P
Laboratory studies						
Prey size	104	PC1	LEN	3.70	9.74	0.002
			RA	-0.213	13.00	<0.001
			DENS	-3.51×10^{-4}	0.03	0.854
		PC2	LEN	1.20	2.56	0.113
			RA	-4.52×10^{-2}	1.36	0.246
			DENS	-1.03×10^{-4}	0.01	0.924
Prey species	78	PC1	LEN	3.63	8.11	0.006
			RA	-0.242	8.93	0.004
			DENS	-1.37×10^{-3}	0.59	0.447
		PC2	LEN	-0.374	0.10	0.751
			RA	-9.86×10^{-2}	1.21	0.275
			DENS	3.63×10^{-4}	0.05	0.822
Field studies						
Prey size	43	PC1	LEN	-2.33	31.1	<0.001
			RA	-9.99×10^{-2}	2.50	0.122
			DENS	2.83×10^{-3}	5.92	0.020
			TEMP	3.74×10^{-2}	0.74	0.396
		PC2	LEN	0.970	9.56	0.004
			RA	-0.142	7.41	0.010
			DENS	5.78×10^{-4}	0.58	0.451
			TEMP	-7.34×10^{-2}	5.77	0.021
Prey species	34	PC1	LEN	-1.86	8.61	0.007
			RA	-0.223	5.13	0.031
			DENS	4.09×10^{-3}	7.69	0.010
			TEMP	8.21×10^{-2}	2.24	0.145
		PC2	LEN	-1.35	7.63	0.010
			RA	-3.68×10^{-2}	0.21	0.653
			DENS	-1.75×10^{-3}	1.93	0.175
			TEMP	3.41×10^{-2}	0.65	0.426

of DENS on electivity in the above analyses was consistent with these predictions for the field studies but not for the laboratory studies. We thus decided to analyze the laboratory data more closely. The A_{EQ} treatment in the laboratory experiments provided larvae with equal proportions of the three zooplankton sizes. Using only data for small larvae (<11 mm mean length) from the A_{EQ} treatment, we reconducted the regression of PC1 of the prey size electivity vector against LEN, RA, and DENS. In this analysis, DENS had a significant, negative effect on the electivity of medium and large relative to small prey (ANOVA, $F = 4.57$, df = 1,43, $P = 0.038$). This result was thus in agreement with the predictions arising from the functional response analysis.

The effects of temperature on electivity were examined only in the field studies (Table 5). Regression parameters indicated that selection for larger relative to smaller prey declined with increasing temperature. This effect was significant for the second prey size principal component. Temperature effects on prey species electivity were not significant (Table 5).

Discussion

Functional Response

Our analysis and interpretations of the walleye's functional response must be put in perspective with the characteristics of the model we used. The Holling functional response model predicts that search time (foraging time spent actively locating prey) declines relative to handling time (foraging time spent in pursuit, capture, and ingestion of prey plus a digestive pause before resumption of searching) with increasing prey abundance and that virtually all foraging time is spent in handling when prey are very abundant. The model parameters, C_{max} and D_{90} (or f and a, equations 2 and 3), are assumed constant. However, Abrams (1990) has argued that the parameters vary with prey abundance. Prey abundance may affect the model parameters indirectly through its influence on predator satiation. There is some evidence, for example, that the handling time per unit of prey increases with satiation (Werner 1974; Confer and O'Bryan 1989). If this is the case, then the reliability of

our parameter estimates may depend upon the similarity in satiation levels among our experimental fish. We terminated laboratory trials well before the larval guts became full, and the amount of food in the experimental fish guts was always much lower than that observed in the guts of the Methley pond walleye larvae, which presumably had been feeding continuously for several hours prior to capture. We feel that our results best represent the functional response of unsatiated larvae. Our estimates of C_{max} and D_{90} are thus probably higher than would be expected for continuously feeding fish. Estimates of the consumption rate of walleye larvae based on feeding experiments of much shorter duration (Johnston and Mathias 1994) also suggest that our parameter estimates are lower than would be observed for fish that had just commenced feeding.

Our results illustrated the changes in the walleye functional response that accompany increasing body size. C_{max} increased significantly with walleye mean length, a pattern observed in many other larval fishes (Houde and Schekter 1980; Miller et al. 1992). This corresponds with a rapid increase in the walleye's prey capture success during the same period (Johnston and Mathias 1994). D_{90} increased initially and then declined with respect to walleye mean length. Although our statistical analysis indicated that D_{90} differed significantly between experimental dates, our design did not permit us to distinguish between the effects of mean length and other effects related to experimental date, such as fish stock. We feel that the observed trend between D_{90} and mean length does reflect the true relationship but suggest that it should be reexamined in future studies. If the observed D_{90} – mean length relationship is correct, then the ability of walleye larvae to feed at maximal rates is most limited by zooplankton abundance not at first-feeding but when larvae are ~10–10.5 mm mean length. D_{90} will increase in response to a reduced handling time but decrease in response to improved searching ability (equation 3). This suggests that size-related changes in the feeding ecology of walleye during early development initially favour improvements in handling prey (increasing C_{max}) over improvements in locating prey. Changes in feeding ability during early development may result from, or simply be correlated with, increasing body size. Certainly, swimming speed increases with larval length (reviewed by Hunter (1981) and Blaxter (1986)) and has a major influence on both search rate and handling time. Physical changes in musculature, swim bladder inflation, and fin structure also improve swimming ability (reviewed by Blaxter 1986) and changes in eye structure increase the distance at which prey can be detected (Hairston et al. 1982; Wahl et al. 1993). Experience also increases over time and may be as important as physical changes in improving the searching and handling efficiencies of larval fish (reviewed by Hughes et al. 1992).

The functional response of walleye larvae also varied with respect to prey size. C_{max} for first-feeding walleye was highest when feeding on small prey and lowest when feeding on large prey and this trend reversed with increasing walleye size. Our analysis indicated that this interaction between the effects of walleye size and prey size on C_{max} was not statistically significant. However, the trend is consistent with our expectations. Larger zooplankton should become easier to handle relative to smaller zooplankton as walleye grow because increasing gape and gill-raker spacings favour the capture of increasingly larger prey. We suspect that this trend would be more pronounced, and perhaps statistically significant, when examined over a wider range of walleye body sizes. Prey size had a stronger effect on D_{90} than on C_{max}. D_{90} was significantly lower for large relative to small prey. Larger prey are easier to detect (Confer and Blades 1975; Confer et al. 1978) and thus the abundance necessary to allow near-maximal rates of consumption would be lower. We expected that walleye larvae of all sizes would be able to detect larger prey more easily than smaller prey and thus, interaction between walleye size and prey size would be negligible. Our results supported this expectation, as D_{90} was higher for small prey than for large prey for almost all walleye mean lengths examined.

Because our zooplankton size treatments varied in species composition, observed differences in the functional response between zooplankton size classes may also reflect differences in prey species among them. Zooplankton of the small size fraction were primarily cyclopoid copepods whereas zooplankton of the large size fraction were mostly daphnids. Copepods and cladocerans utilize quite different predator evasion tactics (Kerfoot et al. 1980), with the latter being much more susceptible to the suction-type intake of fish (Drenner et al. 1978). The capture success of planktivorous fish is thus usually higher when feeding on cladoceran prey (Confer and Blades 1975; Wanzenböck 1992). These and other interspecific differences in prey may have influenced the observed effect of our zooplankton size treatments on the functional response model parameters.

A practical value of functional response analysis has been to provide estimates of the prey abundances necessary to allow fish to feed at maximal rates. Mathias and Li (1982) estimated that the consumption rate of young walleye was maximized at zooplankton abundances of $\geq 100 \cdot L^{-1}$ based on eye-fitted interpretations of their functional response data. Our results indicate that the threshold zooplankton abundance varies with both walleye and zooplankton size. Based on the fitted parameter D_{90}, 9.5- to 10.5-mm walleye larvae would require prey abundances of $200–800 \cdot L^{-1}$ for small zooplankton or $20–300 \cdot L^{-1}$ for large zooplankton to attain 90% of C_{max}. Beyond this walleye size range, threshold zooplankton abundances decline markedly and 13- to 15-mm would probably only require prey abundances as high as $100 \cdot L^{-1}$ for small zooplankton. However, these very high estimates of D_{90} must be viewed with caution. Our experiments were conducted using prey abundances $\leq 180 \cdot L^{-1}$, but D_{90} estimates were sometimes far above these levels. For zooplankton abundances $>180 \cdot L^{-1}$ the functional response model may be inappropriate. For example, cyclopoid copepods (the main component of the S treatment) at abundances $\geq 500 \cdot L^{-1}$ will consume walleye larvae (Hokanson and Lien 1986; T.A. Johnston, personal observation) and under such conditions the walleye consumption rate would obviously decline with increasing prey abundance. Consumption rate may also decline with increasing prey abundance if the fish are confused by seeing many prey items at once (Marcotte and Browman 1986). Such an effect has been observed for first-feeding walleye larvae preying on large zooplankton (J.A. Mathias, unpublished data). The inability of the model to predict the functional response at very high prey abundances would likely only be important under artificial conditions such as in extensive or intensive culture systems. Zooplankton abundances encountered by most walleye larvae in natural waterbodies would probably be within the range

of abundances used in this study. In prairie lakes such as Dauphin Lake or Lake Winnipeg, mean zooplankton abundances at the time of walleye first feeding are in the range of 20–70·L^{-1} and are usually composed of small zooplankters dominated by cyclopoid copepods (Patalas 1975; Friesen and Mathias 1990; Patalas and Salki 1992).

The application of our functional response results to the prediction of walleye feeding under natural conditions must also consider the prey distribution. The functional response model assumes a random distribution of prey organisms, a condition that is easily maintained in laboratory aquaria but that is probably uncommon in natural communities. But the feeding of fish larvae may depend as much upon the variance as the mean of zooplankton abundance. For a given mean zooplankton abundance, feeding success should improve with increasing prey patchiness, reach an optimum, and then decline under conditions of extreme clumping (Vlymen 1977). At low levels, clumping creates localized high-density patches that allow a higher consumption rate. Minor prey clumping would reduce the value of D_{90} but would not affect C_{max}. However, extreme prey clumping creates very high-density patches that may be confusing, or even dangerous (if predacious zooplankton are present), for fish larvae and that are too widely spaced to be easily found. Thus, walleye larvae in natural lakes may be able to feed at higher rates at low prey abundances than would be predicted by our functional response model if prey were moderately aggregated.

Prey Selection

Prey selection by walleye larvae was strongly dependent upon walleye body size. Selection for larger relative to smaller prey and selection for large cladocerans relative to cyclopoid copepods both increased significantly with walleye mean length. Similar trends have been observed in previous studies of walleye larvae (Raisanen and Applegate 1983; Graham and Sprules 1992) and of numerous other species (Miller et al. 1988; Schael et al. 1991). Cyclopoid copepods appear to be an important dietary component and a preferred prey of first-feeding walleye (Spykerman 1974; Fox 1989; Graham and Sprules 1992; this study), but selection shifts rapidly to larger prey. This shift is associated with an increasing mouth gape that allows larger prey to be ingested (Mathias and Li 1982; Graham and Sprules 1992). Walleye have one of the largest gapes relative to their body size among freshwater fish larvae (summarized by Ghan and Sprules 1993) and they will eat larger prey than other larval fishes in the spring ichthyoplankton community (Bulkley et al. 1976; Michaletz et al. 1987). Even walleye as small as 10 mm will consume fish larvae although they are usually only a minor dietary component (Priegel 1969; Bulkley et al. 1976; Li and Ayles 1981).

The negative relationship observed between electivity and relative prey abundance indicates that walleye larvae select most strongly for prey that are relatively uncommon in the zooplankton community. This trend was observed in both laboratory and field studies. Emlen (1966) hypothesized that selection for a prey type would increase with its relative abundance. Foraging studies on guppies, *Poecilia reticulatus*, confirmed the positive influence of relative prey abundance, and the pattern, termed prey switching, was considered to be a more efficient foraging strategy (Murdoch et al. 1975). The "negative switching" exhibited by walleye larvae in this study indicates a strategy to broaden the diet by selecting most strongly for uncommon prey. Mills et al. (1987) noted a similar pattern in zooplanktivorous juvenile yellow perch, *Perca flavescens*, and hypothesized that selection for novel prey reflected nutrient requirements of the fish. Diets may be broader than expected and foraging strategies may deviate from what is considered energetically optimal for a variety of reasons (Westoby 1978; Hart 1986). It is not possible to say from our results whether this foraging pattern is an adaptation to meeting nutrient constraints or simply part of the learning process. If the latter is true, negative switching may be a characteristic of feeding in young fish and/or a characteristic of the early stages of each feeding bout for all fish.

Based on the results of our functional response analysis, we predicted that selection for smaller relative to larger prey would increase with total zooplankton abundance. This trend was significant in our field studies but not in the laboratory studies, with the exception of a specific subset of the data. Optimal foraging theory predicts that selectivity increases with prey abundance (Emlen 1966; MacArthur and Pianka 1966). More recent studies stress predator satiation as the primary determinant of selectivity and consider prey abundance as a correlate of satiation (Bence and Murdoch 1986; Hart and Gill 1992). Changes in electivity with time spent foraging (e.g., Furnass 1979; Confer and O'Bryan 1989) may reflect a similar satiation mechanism. But at least one study has found no effect of prey abundance or predator satiation on prey selection (Mills et al. 1986). We suspect that the laboratory walleye that were food-deprived and then allowed to feed for only a short time were not as strongly selective as the pond walleye whose guts were usually full. However, laboratory and field conditions differed in other respects (Table 3) which may have also influenced the effect of prey abundance on prey selection. Juvenile yellow perch show stronger selection for copepods relative to *Daphnia* sp. with increasing prey abundance (Confer et al. 1990), a trend consistent with our field results. Increasing prey abundance (or predator satiation) may reduce the variance in prey selection as well as change the magnitude of selection. Reductions in the variance of electivity with increasing prey abundance were observed in both the laboratory and field components of this research and were statistically significant in the former.

Temperature has a major influence on food consumption rate in fishes and yet relatively little is known of the effects of temperature on prey selection. In this study, temperature had a significant effect on prey size electivity by walleye larvae under field conditions. Selection for medium relative to small prey declined with increasing water temperature. In more recent laboratory experiments, we found no statistically significant effect of temperature on prey selection by walleye larvae (Johnston and Mathias 1994). However, these experiments were of short duration (<10 min), used larvae that were deprived of food prior to the experiment, and examined a much narrower range of water temperatures (15–22°C). Werner and Hall (1974) hypothesized that increasing diet breadth in response to declining temperature represented an optimal allocation of time given that a fish's search rate will decline with temperature. This suggests that prey selection will become stronger with increasing temperature and is consistent with the findings of this study. The mechanism behind selection for smaller prey by walleye at warmer temperatures may also be related to changes in search rate. An increasing search rate may increase encounter

rates with small prey relative to encounter rates with large prey. An alternative is that temperature might simply be correlated with another variable affecting prey selection, such as light, time of day, or barometric pressure. Light has been shown to influence prey selection. In laboratory feeding experiments with juvenile yellow perch, Mills et al. (1986) observed a decline in the median size of prey consumed and a decline in selection for daphnids relative to cyclopoid copepods with increasing light intensity. However, this trend was not observed in their field studies.

The pattern of prey selection exhibited by walleye larvae may be the result of both predator and prey characteristics. Walleye may actively choose specific prey sizes and/or species that optimize growth. Growth of larval and juvenile fishes has been linked to both prey size (Fox 1989) and prey species (Forney 1966; Hokanson and Lien 1986; Confer and Lake 1987). The importance of prey size alone has been demonstrated in laboratory experiments by feeding fish with pellets of different sizes but identical compositions (Wankowski and Thorpe 1979; Tabachek 1988). However, the effect of prey species on growth is often difficult to separate from the size effect, as different species are naturally of different sizes. Patterns in prey selection may also reflect the relative ease with which different prey species can be found, captured, and ingested.

Our results have several implications for understanding the feeding ecology of walleye larvae in natural waterbodies. Walleye show strong selection for relatively large prey early in their development. However, in many lakes, and particularly those with abundant zooplanktivorous fishes, the zooplankton community is dominated by smaller organisms such as cyclopoid copepods and bosminids, and large cladocerans are relatively rare (Friesen and Mathias 1990; Mazumder et al. 1992; Patalas and Salki 1992). A lack of the preferred, larger zooplankton in such lakes may force walleye to become benthivorous or piscivorous at a smaller size than they would in lakes with abundant large zooplankton. A scarcity of larger bodied zooplankton has been hypothesized as a major factor limiting growth and survival of other larval fishes (Crowder et al. 1987). If the walleye's strong selection for relatively uncommon prey reflects a nutrient constraint, feeding and growth may be limited in zooplankton communities that lack specific prey types. However, at present, little is known of the nutrient requirements of freshwater fish larvae or the nutrient composition of their zooplankton prey. The significance of changes in prey selection with respect to prey abundance and water temperature is not readily apparent. Growth rates of walleye larvae in extensive culture systems increase with both prey abundance and water temperature (Johnston et al. 1992), presumably because of increased food conversion efficiency and/or food consumption. Increased selection for relatively smaller prey may thus be an adaptation that increases food conversion efficiency when large volumes of food are passing through the digestive tract and/or allows a higher consumption rate.

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Relationships between Mercury Concentrations in Walleye (*Stizostedion vitreum*) and Northern Pike (*Esox lucius*): Implications for Modelling and Biomonitoring

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Site-specific and interlake models are developed and tested for predicting mercury levels in walleye (*Stizostedion vitreum*) and northern pike (*Esox lucius*). The models are based on empirical relationships from coexisting fish in waters impacted by atmospheric loadings, contaminated sediments, and new reservoir formations. The sizes of fish used in the model (39-cm walleye and 55-cm northern pike) are typical of those caught by sport and commercial fishermen. The relative importance of a number of environmental factors to the accumulation of mercury in coexisting populations was examined by regression of the Biotic Mercury Partitioning Index (BMPI: mercury in species 1 (39-cm walleye)/mercury in species 2 (55-cm northern pike)) on pH, alkalinity, dissolved organic carbon, and other variables that have been identified as important to the accumulation of mercury in either or both species. No species-specific differences in mercury bioavailability were identified. We postulate that development of other biotic relationships on a site-specific, watershed, or broader geographic basis will assist in attempts to model mercury in aquatic ecosystems and reduce sample requirements for biomonitoring programs.

Des modèles spécifiques au site et interlacustres ont été mis au point et soumis à des essais pour la prévision des concentrations de mercure chez le doré (*Stizostedion vitreum*) et le grand brochet (*Esox lucius*). Ces modèles sont basés sur des relations empiriques provenant de poissons coexistant dans des eaux qui subissent les effets de charges atmosphériques, de sédiments contaminés et de la formation de nouveaux réservoirs. La taille des poissons utilisés pour le modèle (39 cm dans le cas du doré et 55 cm pour le grand brochet) est celle qui correspond généralement aux prises des pêcheurs sportifs et commerciaux. L'importance relative d'un certain nombre de facteurs environnementaux pour l'accumulation du mercure chez les populations coexistantes a été examinée par analyse de régression de l'indice de partage du mercure biotique (IPMB : mercure chez l'espèce 1 (doré de 39 cm)/mercure chez l'espèce 2 (grand brochet de 55 cm) sur le pH, l'alcalinité, le carbone organique dissous et d'autres variables, paramètres qui influent fortement sur l'accumulation du mercure chez l'une ou l'autre des espèces ou chez les deux. Aucune différence spécifique entre les deux espèces n'a été constatée pour ce qui est de la biodisponibilité du mercure. Nous supposons que l'élaboration d'autres relations biotiques, couvrant un site spécifique, un bassin hydrographique ou une zone géographique plus vaste permettra de faciliter les essais de modélisation du mercure dans les systèmes aquatiques et de réduire les besoins en échantillons pour les programmes de biosurveillance.

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Mercury remains a world health concern. Fish are of particular interest because they are typically the major source of methyl mercury, a potent neurotoxin, for humans and other mammals. Unacceptably high mercury levels are reported in fishes inhabiting waters in the United States (Sorensen et al. 1990), Canada (Wren et al. 1991), and Scandinavia (Lindqvist et al. 1984; Håkanson et al. 1988). Elevated concentrations in fish have been attributed to a variety of causes, including point-source loadings to waters (Armstrong and Hamilton 1973), contaminated sediments (Allan et al. 1984), atmospheric loadings (Mierle 1990; Sorensen et al. 1990), reservoir formation (Bodaly et al. 1984; Stokes and Wren 1987; Jackson 1991), and increasing acidification of lakes and watersheds (Richman et al. 1988).

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Although public health restrictions on the consumption of mercury-contaminated aquatic organisms are in place, it is difficult to provide official health advisories for all affected waters because large numbers of samples are required to define mercury levels in a given aquatic community, and concentrations in similar species vary geographically.

Establishing such public health advisory (biomonitoring) programs involves a number of decisions regarding (1) target organisms, (2) sample size, (3) sampling frequency, and (4) sampling locations. These decisions are often subjective, since in the vast majority of cases there is little or no information on site-specific factors governing the distribution and accumulation of mercury. Mercury concentrations in the aquatic community in a single water body can vary by over three orders of magnitude (Armstrong and Hamilton 1973), and within a single species by one order of magnitude (Parks et al. 1991a).

A large number of factors influence the accumulation of mercury in fish, including age, size, metabolism, water temperature, sex, and mercury concentrations and speciation in both water and food (Huckabee et al. 1979; Richman et al. 1988). Because many of these variables change significantly within a watershed, biological data collected for any given waterbody are frequently of limited utility for extrapolation. Mercury concentrations, for example, in similar fish can vary by a factor of 2–5 among nearby lakes. On a broader geographic basis, differences of 50-fold are not uncommon (Guide to Eating Ontario Sport Fish 1988). Overall, this site-specific and geographic variability produces large amounts of new data with each mercury issue that arises.

Progress in understanding this variability by modelling mercury accumulation in fish has been encouraging (e.g. Johnston et al. 1991; Diamond and McKay 1992; Harris and Snodgrass 1992; Hudson et al. 1992; Rodgers 1992). However, regardless of the ecosystem modelling approach(s) employed — bioenergetic, mechanistic, steady state, dynamic equilibrium, rate limiting, or other — the precision associated with most models is not particularly satisfying.

The uncertainty over the mode of mercury accumulation in fish also extends to the larger aquatic community. Much of this uncertainty arises from a lack of quantitative data for both inorganic mercury and the highly bioavailable methyl mercury in aquatic ecosystems. Owing in part to this lack of data, there is a growing number of reports correlating water chemistry parameters and other environmental variables with mercury concentrations in various species of fish or other aquatic biota (e.g., sunfish: Wren et al. 1983; perch: Cope et al. 1990; smallmouth bass: McMurtry et al. 1989). However, we can only infer cause and effect, or speculate on a number of possible causes including physiological changes to the organism permitting different uptake rates, increases in mercury concentrations in the environment, changes in the speciation and bioavailability of mercury present, and changes in food webs. Many studies have suggested that a number of factors, such as water pH and dissolved organic carbon, have important effects on the accumulation of mercury in different species; however, the quantitative importance of these findings is rarely consistent and can be contradictory, even for the same species.

There are over 50 000 publications dealing with various components of the mercury cycle (Björnberg et al. 1988), but relatively few papers have examined in detail relationships between mercury concentrations among different species of fish or other organisms (e.g., MacCrimmon et al. 1983; Parks 1988; Allard and Stokes 1989; Sorensen et al. 1990; Jackson 1991; Lundqvist et al. 1991a; Wren et al. 1991). Most of these studies focus on a restricted geographic area and typically explain mercury concentrations by predator–prey interactions. This paper takes a more holistic approach. Here, we synthesize and examine relationships between mercury concentrations in two coexisting species of fish from systems affected by a variety of mercury sources: contaminated sediments, atmospheric loadings, and new reservoirs. From this synthesis, we believe these relationships can be used to (1) reveal relationships useful for modelling community responses to changes in mercury exposure, (2) increase the efficiency of biomonitoring programs designed to protect public health, (3) provide a rationale for selecting better national and international bioindicators, and (4) provide a conceptual framework to examine how water quality and other environmental factors affect the bioavailability of mercury to various organisms.

Northern pike (*Esox lucius*) are widely distributed in the United States, Canada, Scandinavia, and Eurasia (Scott and Crossman 1973) and have been extensively used as bioindicators of mercury (Johnels et al. 1967; Bishop and Neary 1976; Wren et al. 1991). Walleye (*Stizostedion vitreum*) are members of the perch family, which is also distributed over much of North America and Eurasia, but have been less commonly used as mercury bioindicators. A relationship between mercury concentrations in northern pike and walleye was first observed by Bishop and Neary (1976). More extensive studies have since been conducted by Sorensen et al. (1990) in Minnesota and Wren et al. (1991) in Ontario. Site-specific relationships for northern pike and walleye from two lakes in northwestern Ontario recovering from chlor-alkalai discharges have been presented by Parks et al. (1991a), and walleye – northern pike relationships in reservoirs and lakes of northern Manitoba were analysed by Jackson (1991).

Methods and Materials

Data Sources: Pristine Lakes, New Reservoirs, and Historically Contaminated Waterbodies

Data were drawn from studies of three different mercury issues where mercury levels in fish warrant consumption guidelines. Data were obtained for 42 "pristine" (no known point sources of mercury) in northern Minnesota, USA (Sorensen et al. 1990). In addition, data were obtained for nine reservoirs associated with hydroelectric generation on the Churchill River diversion in northern Manitoba, Canada (Green 1986). As will be shown below, these reservoirs are some of the most extensively studied worldwide. Finally, data were obtained for four lakes in the Wabigoon/English/Winnipeg River system in northwestern Ontario, Canada, a watercourse recovering from sediments contaminated with mercury discharged from a chlor-alkalai plant at Dryden, Ontario, during the 1960's (Allan et al. 1984; Table 1). The Wabigoon/English/Winnipeg River system is one of the more intensively studied watercourses with regard to the cycling, effects, and fate of mercury introduced from a point source.

Standardization for Size

In order to compare walleye and northern pike, data on the length, weight, age, and mercury concentrations of

individual fish are required. Correlations between mercury and length, weight, or age are commonly observed for northern pike and walleye (Scott 1972; Fagerström et al. 1974, 1975; Bishop and Neary 1976; Green 1986; Lathrop et al. 1990; Sorensen et al. 1990; Wren et al. 1991). As length, weight and age parameters are intercorrelated, we standardized fish for length to facilitate comparisons because there were more length data available to compute regressions. Further, length is believed to be a more precise predictor than weight (Scott 1972; Bishop and Neary 1976). Additionally, Parks et al. (1991a) observed significant correlations between mercury concentration and length for fish of the same age and concluded that size standardization was required for intersite comparisons. Some researchers have reported poor, no, or even negative relationships between mercury concentrations in walleye or northern pike and length (see discussion in Jackson 1991). These results can occur in aquatic systems where net changes in the supply of mercury over time scales of years are small, and with an insufficient sample size range. Most commonly, results for the smaller organisms are unavailable (Parks et al. 1991b).

Fish were standardized from regressions using log-transformed variables (Scott 1972; Bishop and Neary 1976; Sorensen et al. 1990), when there was a significant relationship between log mercury and log length ($p < 0.05$) and a sample size of at least 10. Some researchers have used a semilogarithmic approach (McMurtry et al. 1989; Wren et al. 1991), i.e., log mercury versus length. For Ontario lakes, there were no significant differences between correlation coefficients for log–log and log–linear regressions for walleye and northern pike ($p < 0.05$), when we used the test by Sokal and Rohlf (1981) to compare coefficients. Others have found that untransformed variables have provided satisfactory results (Brouard et al. 1989; Lathrop et al. 1991). Regressions using untransformed variables may be adequate for samples composed largely of mature northern pike or walleye but are likely inappropriate for whole-population approaches.

Regardless of which mathematical approach is used, differences in the slopes of the relationships should be noted, both between species and between lakes (Table 1) (Scott 1972; Green 1986; Lathrop et al. 1990). These differences restrict the discussion to the sizes of the fish compared, and the conclusions may not necessarily apply to whole populations unless whole-population effects are specifically identified.

Because of differences in the slopes of the regression lines, we did not utilize a covariance approach to normalize for size but rather standardized mercury concentrations from individual regressions (Parks et al. 1991b). The standard lengths selected for walleye and northern pike (39 and 55 cm, respectively) were based on a statistical analysis of fish from lakes in northern Minnesota performed by Sorensen et al. (1990), who observed that these sizes produced the highest correlation between mercury concentrations in each species. Poorer correlations at higher sizes may be due to effects of sexual dimorphism. Mature male and female northern pike, for example, accumulate mercury at different rates (Johnels 1967; Phillips et al. 1980). The sizes selected are similar to (a) the average size of fish obtained in the Ontario Sportfish Contaminant Monitoring Program (41 cm for walleye and 54 cm for northern pike) and used by Wren et al. (1991) and (b) the sizes selected for data analysis used in the Churchill River diversion (40 cm for walleye and 55 cm for northern pike) (Green 1986), but are smaller than the 50-cm walleye and 60-cm northern pike used by Bishop and Neary (1976) and Parks et al. (1991a) for lakes in northwestern Ontario.

Role of Environmental Variables on Mercury Accumulation

A number of environmental correlations between mercury concentrations in fish and environmental factors have been identified for either or both species (Håkanson 1980; Håkanson et al. 1988; Helwig and Heiskary 1985; Mannio et al. 1986; Lathrop et al. 1989, 1990; Sorensen et al. 1990; Weiner et al. 1990; Jackson 1991; Rask and Metsala 1991; Wren et al. 1991), but there remains substantial uncertainty, inconsistency, and confusion over the meaning, importance, and general applicability of many of these correlates. For example, mercury in walleye was correlated inversely ($p < 0.05$) with pH, alkalinity, calcium, and conductivity in Wisconsin lakes (Lathrop et al. 1989), but not in Ontario lakes (Wren et al. 1991) (Table 2). In another study of Ontario walleye, however, Scheider et al. (1979) concluded that accumulation of mercury was related to alkalinity. Different environmental variables have been identified as important in the accumulation of mercury in northern pike and walleye in Ontario lakes, even for coexisting populations (Wren et al. 1991) (Table 2).

Common approaches to the evaluation of the species-specific influence of environmental variables on mercury accumulation in northern pike or walleye involve regression, multiple regression, stepwise multiple regression, and/or factor analysis using an assortment of water quality and biophysical lake characteristics. As an alternative, we created a Biotic Mercury Partitioning Index (BMPI) by dividing the mercury concentration in one species (the standard walleye) by that in another (northern pike) for fish from the same lake. We then correlated the BMPI with environmental variables that were known to correlate with the mercury concentrations in populations of each species in Ontario lakes. If an environmental variable were more important to one species than the other, a significant correlation could be expected between the BMPI and the variable of interest. We believe that this approach provides additional insight into the relevance of various variables where correlations between mercury in coexisting species and environmental variables may be low, statistically significant, but different between species — an occurrence commonly observed in studies of this type (Lathrop et al. 1991).

This type of ratio is not believed to be biased by spurious correlation because the numerator (mercury in 39-cm walleye) and the denominator (mercury in 55-cm northern pike) are colinear and the intercept does go through zero (see Jackson and Somers 1991 for concerns regarding spurious correlations).

Results

Relationships between Mercury Levels in Walleye and Northern Pike

Relationships between mercury levels in walleye and northern pike are evident not only within a single lake but also among various groups of lakes (Tables 3 and 4;

TABLE 1. Mercury concentrations and other selected characteristics of Clay, Ball, Separation, and Tetu lakes walleye and northern pike. (*$p < 0.05$; **$p < 0.01$).

		Mercury (μg/g)		Length (cm)		Weight (g)		log Hg, on log length[a]		
Year	*n*	Mean	Range	Mean	Range	Mean	Range	AO	AL	r^2
				Clay Lake walleye, 1970–87						
1970	274	12.1	1.20–24.0	41.6	22.5–53.0	954	370–1900	−2.969	1.453**	0.235
1971	24	8.71	1.82–14.2	42.6	33.0–50.0	1064	454–1850	−0.481	0.680	0.020
1975	88	6.47	2.54–11.59	44.6	30.5–53.5	1056	315–1675	−4.738	1.729**	0.415
1976	50	7.83	4.50–12.1	49.8	40.8–59.4	1283	482–2013	−2.415	1.141**	0.253
1977	30	6.97	2.90–9.9	50.3	42.0–59.0	1321	910–1920	−3.427	1.365**	0.267
1978	7	6.87	3.93–11.2	43.4	29.0–59.0	1019	250–2100	−3.201	1.355**	0.848
1979	51	5.90	3.06–9.9	47.0	32.7–62.2	1068	340–2220	−3.878	1.463**	0.777
1980	16	8.64	3.67–13.1	55.5	41.3–63.0	1672	752–2150	−8.531	2.654**	0.915
1981	18	6.74	3.30–11.0	56.1	44.6–65.2	1764	900–2400	−11.39	3.290**	0.848
1982	20	4.36	1.20–8.70	51.6	28.0–67.5	1605	200–3825	−6.368	1.972**	0.810
1983	21	2.55	1.6–7.0	36.5	27.5–71.5	677	200–3825	−2.906	1.0576**	0.538
1984	22	2.79	1.6–4.5	36.1	20.4–56.3	645	70–1800	−2.290	0.923**	0.829
1985	20	2.25	1.10–4.9	41.1	22.6–58.5	914	100–2450	−4.314	1.370**	0.767
1986	20	2.11	0.92–3.3	41.0	25.5–63.0	856	220–3000	−4.665	1.452**	0.788
1987	20	2.33	1.38–4.33	38.6	26.5–64.5	683	250–3000	−3.662	1.229**	0.733
				Clay Lake northern pike, 1971–87						
1971	25	9.09	3.79–14.9	50.8	36.0–62.0	974	312–1870	−2.808	1.264**	0.259
1975	116	5.43	1.3–11.30	54.9	34.0–81.0	1292	280–3610	−4.482	1.524*	0.275
1976	51	5.84	3.60–13.0	65.0	50.1–86.2	1793	879–4423	−3.071	1.152**	0.325
1977	30	4.83	2.20–8.41	61.3	45.0–76.4	1567	500–3500	−4.357	1.433**	0.252
1978	20	4.67	0.97–8.5	58.7	37.0–73.5	1411	300–2500	−7.383	2.176**	0.644
1979	24	3.87	0.98–7.02	60.4	45.0–76.4	1567	500–3500	−9.684	2.276**	0.638
1980	17	2.44	0.85–5.5	49.2	30.0–73.2	846	125–2400	−5.020	1.478*	0.264
1981	17	2.34	0.63–5.71	55.7	34.0–87.0	1764	900–2400	−4.669	1.359**	0.524
1982	21	2.32	0.44–4.70	53.5	16.5–84.0	1096	21–4200	−3.824	1.147**	0.364
1983	20	2.34	0.92–3.80	47.3	30.0–56.5	698	150–1150	−4.933	1.489**	0.559
1984	20	2.40	1.9–4.3	55.1	31.8–69.5	1896	200–2000	−2.703	0.8843*	0.344
1985	20	1.63	0.64–2.90	57.0	40.2–72.2	1366	450–2950	−4.328	1.176*	0.184
1986	6	2.30	1.4–3.09	62.7	54–70	1908	1050–3000	−7.341	1.967	0.345
1987	12	2.15	1.3–3.13	55.4	41.5–64.2	1108	450–1550	−1.707	0.605	0.067
				Ball Lake walleye, 1971–86						
1971	20	2.88	0.80–6.22	41.1	31.0–50.0	921	340–1701	−2.040	0.810	0.044
1974	50	2.45	0.94–4.42	46.0	34.3–58.4	1189	397–2466	−2.665	0.810*	0.045
1975	58	1.584	0.41–4.26	44.3	22.5–61.5	936	85–2875	−2.409	0.710**	0.047
1976	48	2.233	0.28–4.70	36.6	27.5–48.0	578	200–1400	−10.060	2.964**	0.346
1977	30	1.527	0.67–3.10	44.2	35.0–59.0	638	200–190	−4.605	1.311*	0.267
1978	20	2.13	0.71–3.50	42.9	23.5–58.0	901	114–1800	−2.123	0.753*	0.214
1979	20	1.92	0.94–3.60	45.8	26.0–62.5	1055	113–2268	−2.546	0.828*	0.361
1980	20	1.70	0.96–2.45	47.9	37.0–58.0	1125	450–1800	−3.225	0.963*	0.144
1981	20	1.172	0.25–2.60	42.0	25.0–57.0	934	113–1913	−6.821	1.841**	0.558
1982	20	1.234	0.50–2.10	44.2	30.0–59.0	1045	200–2200	−6.060	1.638**	0.657
1983	30	1.110	0.41–2.6	44.4	24.0–67.3	1018	150–3300	−5.346	1.423**	0.658
1984	20	1.323	0.49–2.50	44.0	24.2–69.8	1013	150–3400	−3.986	1.111**	0.440
1985	20	0.980	0.44–2.50	42.1	26.0–51.3	995	200–18000	−5.906	1.556**	0.371
1986	20	1.365	0.73–2.27	48.9	40.0–62.1	1450	650–3300	−5.794	1.561**	0.419
				Ball Lake northern pike, 1971–86						
1971	20	5.53	1.38–14.9	58.7	48.0–75.0	1517	765–3572	−7.666	2.269*	0.204
1974	32	2.95	0.54–7.95	67.6	50.8–82.6	2314	327–4308	−8.470	2.227*	0.140
1975	42	4.02	0.89–10.7	62.1	43.0–81.6	1585	129–4350	−6.113	1.781**	0.289
1976	49	2.95	0.38–9.40	52.3	29.0–92.5	1146	190–6895	−8.111	2.252**	0.383
1977	27	2.85	0.52–7.50	65.9	48.0–100	1841	500–3378	−7.733	2.054**	0.381
1978	20	3.14	1.10–5.20	63.8	45.5–84.0	1609	507–3378	−7.787	2.124**	0.473

TABLE 1. (*Continued*)

Year	*n*	Mercury (μg/g) Mean	Mercury (μg/g) Range	Length (cm) Mean	Length (cm) Range	Weight (g) Mean	Weight (g) Range	log Hg, on log length[a] AO	log Hg, on log length[a] AL	r^2
1979	20	2.13	0.60–5.43	67.8	50.0–84.0	1885	737–3856	−3.938	1.076	0.052
1980	20	2.50	0.88–4.70	70.4	54.0–98.0	2178	900–7800	−7.187	1.878*	0.183
1981	10	1.52	0.36–7.00	58.8	44.0–90.0	1676	563–4050	−14.59	3.578**	0.745
1982	20	1.65	0.47–3.80	60.9	31.0–97.0	1785	200–5600	−7.190	1.833*	0.649
1983	20	1.50	0.44–2.60	61.3	36.8–84.5	1585	300–4000	−7.117	1.808**	0.492
1984	20	1.63	0.33–3.10	59.8	43.8–85.5	1388	460–4550	−9.300	2.369**	0.411
1985	20	1.53	0.31–5.1	65.2	46.0–96.3	2565	700–89000	−9.23	2.278**	0.525
1986	17	1.4	0.97–2.66	62.8	56.2–78.0	1838	850–3500	−6.630	1.499**	0.134
					Separation Lake walleye, 1971–87					
1974	50	2.77	0.76–4.51	44.4	36.8–58.4	1019	652–2183	−7.332	2.187**	0.339
1975	51	2.99	1.18–4.58	44.5	24.5–55.0	885	160–1550	−5.5540	1.743**	0.578
1976	48	2.23	0.28–4.70	36.6	27.5–48.0	578	200–1400	−10.06	2.964**	0.426
1977	30	2.82	0.79–8.60	43.2	34.5–60.0	764	167–2152	−1.6931	0.771	0.065
1979	20	2.61	1.48–4.98	45.6	38.5–60.0	958	567–2041	−4.409	1.395**	0.294
1980	19	1.95	0.80–3.24	44.7	32.0–62.0	987	300–2100	−2.109	0.718**	0.081
1981	20	1.22	0.54–4.50	38.3	22.5–60.0	1000	225–2475	−5.268	1.471**	0.608
1982	23	1.89	0.71–3.10	44.0	20.0–85.0	1013	100–3000	−4.171	1.261**	0.759
1983	29	1.62	0.36–2.80	45.8	21.1–77.4	1200	100–5400	−5.834	1.653**	0.672
1984	20	1.72	0.65–2.70	44.9	27.6–61.5	983	200–2200	−4.119	1.2143**	0.433
1985	20	1.66	0.84–2.80	46.9	37.4–70.9	1205	500–4000	−6.071	1.70169**	0.771
1987	20	0.90	0.30–3.13	37.6	25.5–73.0	682	150–4700	−6.412	1.706**	0.447
					Separation Lake northern pike, 1971–87					
1971	20	5.09	1.42–14.8	56.9	42.0–76.0	1474	510–4110	−3.925	1.348**	0.180
1974	50	2.96	0.81–6.52	62.1	41.9–80.0	1794	510–3968	−3.194	1.005	0.066
1975	29	2.84	0.91–5.89	58.5	36.6–81.3	1283	400–3820	−6.546	1.841**	0.478
1976	49	2.95	0.38–9.40	52.3	29.0–92.5	1146	190–6895	−8.111	2.252**	0.383
1977	24	2.47	0.75–5.00	59.1	48.5–73.0	1172	405–2270	−6.896	1.890**	0.225
1979	20	1.91	0.83–4.53	57.0	43.0–78.0	1145	397–3176	−6.046	1.636	0.371
1981	20	1.42	0.20–3.60	57.0	27.5–87.5	1783	113–6300	−7.859	1.997**	0.718
1982	22	2.25	0.50–4.30	69.7	37.5–98.0	2505	300–6500	−7.118	1.857**	0.683
1983	20	1.66	0.56–4.20	64.8	30.2–82.4	1898	200–3500	−6.162	1.577**	0.619
1984	22	1.88	0.62–3.00	65.6	43.5–85.7	1852	550–4400	−5.273	1.395**	0.600
1985	20	1.64	0.63–5.80	64.7	46.0–78.5	2035	600–4700	−7.295	1.8434**	0.300
1987	11	1.07	0.50–1.69	57.2	49.5–67.0	1118	750–1850	−10.128	2.505*	0.448
1987	20	0.90	0.30–3.13	37.6	25.5–73.0	682	150–4700	−6.412	1.706**	0.447
					Tetu Lake walleye, 1974–87					
1974	50	1.45	0.40–2.70	44.2	35.6–55.9	1096	510–2013	−7.108	1.955**	0.267
1975	50	1.86	0.58–3.98	46.2	20.5–66.5	1045	80–3050	−5.456	1.571**	0.629
1976	48	1.65	0.65–3.90	37.3	20.5–51.0	632	90–1550	−4.540	1.381**	0.498
1977	30	1.43	0.48–6.00	40.6	21.0–76.0	751	58–40965	−4.839	1.361**	0.391
1978	20	1.07	0.33–2.30	38.8	21.0–64.0	672	57–2508	−4.628	1.261**	0.463
1979	20	1.07	0.36–2.23	40.4	32.0–60.0	651	284–1680	−4.550	1.230**	0.192
1980	19	0.80	0.43–1.13	47.7	41.0–58.0	1037	700–1800	−8.954	2.249**	0.394
1981	20	0.75	0.26–1.50	44.9	30.0–57.5	996	225–2250	−8.300	2.092**	0.693
1982	20	0.75	0.32–2.30	38.3	23.0–56.0	730	100–1900	−5.656	1.452**	0.758
1983	22	1.21	0.65–1.80	51.2	41.5–63.0	1448	750–2500	−4.672	1.227*	0.217
1984	26	0.82	0.40–1.50	40.4	23.6–63.6	764	120–2650	−4.780	1.225**	0.468
1985	20	0.63	0.21–1.50	45.5	24.6–65.1	1288	150–2000	−7.985	1.934**	0.521
1986	13	0.62	0.24–1.86	44.2	33.0–63.5	1142	360–2900	−11.06	2.761*	0.642
1987	20	0.79	0.38–1.42	36.7	24.3–59.0	583	100–2700	−3.2946	0.833*	0.211
					Tetu Lake northern pike, 1974–86					
1974	48	1.74	0.22–6.51	55.9	39.4–85.1	1635	482–6208	−15.34	3.868**	0.564
1975	32	2.87	0.99–10.3	63.5	46.0–97.3	1919	530–7500	−8.104	2.172**	0.508
1976	47	2.23	0.92–6.20	50.9	39.5–79.0	995	420–3750	−8.076	2.241**	0.603

TABLE 1. (*Concluded*)

Year	*n*	Mercury (μg/g) Mean	Mercury (μg/g) Range	Length (cm) Mean	Length (cm) Range	Weight (g) Mean	Weight (g) Range	log Hg, on log length[a] AO	log Hg, on log length[a] AL	r^2
1977	30	1.93	0.24–5.80	56.5	36.0–79.0	1132	298–3178	−4.839	3.014**	0.391
1978	20	1.56	0.63–4.00	59.4	52.0–71.0	1202	825–2028	−9.908	2.510	0.179
1979	20	1.32	0.66–2.05	58.9	51.0–74.0	1236	794–2495	−0.170	0.100	0.001
1980	19	1.08	0.61–2.59	66.6	58.0–77.0	1805	900–3000	0.032	−0.004	0.000
1981	19	0.93	0.26–2.30	66.2	42.5–1055	2214	450–7425	−7.491	1.741**	0.292
1982	20	1.17	0.54–2.20	66.4	52.0–1010	1810	700–6500	−5.307	1.289*	0.196
1983	21	1.10	0.38–2.70	67.1	28.8–103	2310	150–7300	−4.653	1.112**	0.389
1984	26	1.03	0.44–2.30	65.6	521–87.26	1890	800–4300	−7.481	1.772*	0.255
1985	20	0.38	0.23–0.65	52.5	44.4–69.4	1090	700–2300	−8.909	1.993**	0.442
1986	20	0.63	0.31–1.22	58.8	51.0–71.0	1600	1000–3000	−4.806	1.058*	0.235
1987	15	1.00	0.27–2.01	57.2	37.3–82.4	1405	300–3200	−6.931	2.253**	0.700

[a]AO = intercept; Al = slope.

TABLE 2. Comparison of some significant environmental correlations ($p < 0.05$) with mercury in walleye and northern pike in Minnesota, Wisconsin, and Ontario lakes. −, negative correlations; +, positive correlations, 0, no significant correlation; blank, not measured or reported.

Environmental variable	Minnesota: Lakes with walleye[a]	Minnesota: Lakes with northern pike[a]	Wisconsin: Lakes with walleye[b]	Wisconsin: Lakes with walleye[c]	Ontario: Lakes with walleye[d]	Ontario: Lakes with northern pike[d]	Ontario: Lakes with only walleye or northern pike: Walleye[d]	Ontario: Lakes with only walleye or northern pike: Northern pike[d]	Ontario: Lakes with only walleye or northern pike: Northern pike[e]	Ontario: Lakes with only walleye or northern pike: BMPI[f]
Water quality										
pH	−	−	−	−	0	−	0	−	−	0
Alkalinity	0	−	−	−	0	−	0	−	0	0
Ca	0	−	−	−	0	−	0	0	0	0
Conductivity			−		0	−	0	0	0	0
Al	+	+			+	0	+	+	+	0
Total P					0	0	0	0	0	0
DOC					+	+	+	+	+	0
SO_4					+	0	0	0	0	0
Cl					−	0	0	0	0	0
TKN					−	0	0	0	0	0
N					+	0	0	0	0	0
Fe					+	0	+	+	+	0
Mg					0	−	+	+	+	0
Secchi					−	0	−	−	−	0
MEI					0	−	0	0	0	0
Physical										
Latitude					+	−	0	0	0	0
Area				−	−	0	−	0	0	0

[a]Helwig and Helskary (1985), northern pike, 20–25 in.; walleye, 18–20 in.
[b]Lathrop et al. (1989).
[c]Wiener et al. (1990).
[d]Wren et al. (1991).
[e]Calculated from data in Muncaster et al. (1989).
[f]BMPI Hg for 39-cm walleye/Hg for 55-cm northern pike.

Fig. 1–3). There are highly significant correlations between mercury in standardized northern pike and walleye in Clay, Ball, Separation, and Tetu lakes in the Wabigoon/English/Winnipeg River system (Fig. 1; Table 3). Log transformations of the data increased r^2 values at one site only. At three of the lakes, r^2 values exceeded 0.90; Clay Lake was notably lower at 0.79. Site-specific differences in slopes are evident: Clay Lake in the Wabigoon system had the highest (1.15), followed by Ball and Separation lakes in the English system (0.88–0.95), and then Tetu Lake at the Winnipeg River confluence (0.63). From the differences among these slopes, northern pike appear more efficient than walleye at accumulating mercury with distance downstream of the historical point source. The *y*-intercepts were not significantly different from 0 at the 95% confidence level for Clay, Ball, and Separation lakes (Table 3).

A significant regression is also evident when all data for the entire Wabigoon/English/Winnipeg River system are pooled ($r^2 = 0.92$) (Fig. 2; Table 4). Again there was little improvement in r^2 by log transformation of variables.

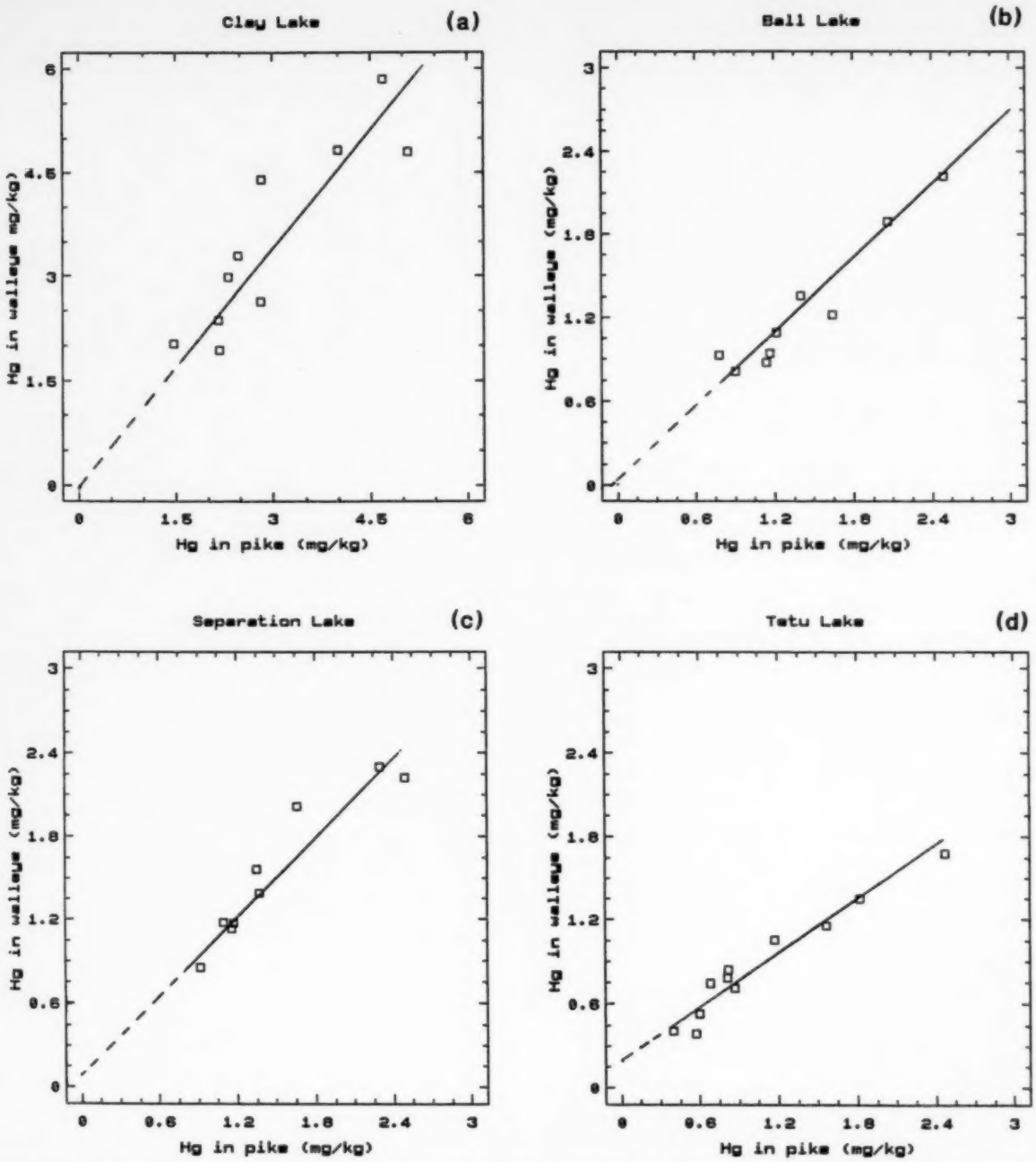

FIG. 1. Relationship (geometric mean regression) between mercury concentrations in walleye (39 cm) and northern pike (55 cm) for four lakes in the Wabigoon/English/Winnipeg River system. Each point represents data for one year. (a) Clay Lake, $r^2 = 0.79$; (b) Ball Lake, $r^2 = 0.92$; (c) Separation Lake, $r^2 = 0.90$; (d) Tetu Lake, $r^2 = 0.96$. All regressions are significant at $p < 0.001$.

The relationships between mercury in standardized walleye and northern pike for the Churchill River diversion is also highly significant ($p < 0.001$) (Fig. 3; Table 4) but has the lowest r^2 (0.47) of any relationship presented.

When all data for groups of lakes in different regions are plotted (Fig. 4), a highly significant relationship is evident. The relationship spans a wide range of concentrations from approximately 0.1 to 6 mg/kg, exceeding individual ranges for relationships reported by Sorensen et al. (1990), Parks et al. (1991a), and Wren et al. (1991). There is little difference in r^2 values for the linear regression ($r^2 = 0.91$) as compared with a log–log regression ($r^2 = 0.93$), but log

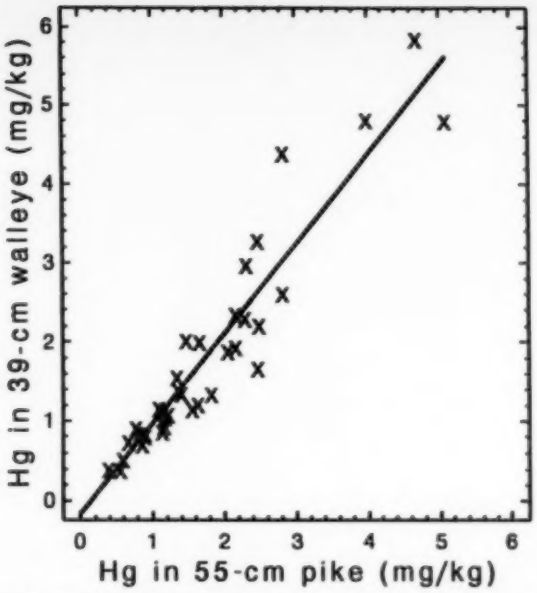

FIG. 2. Relationship (geometric mean regression) between mercury concentrations in walleye (39 cm) and northern pike (55 cm) for the entire Wabigoon/English/Winnipeg River system ($r^2 = 0.87$, $p < 0.001$).

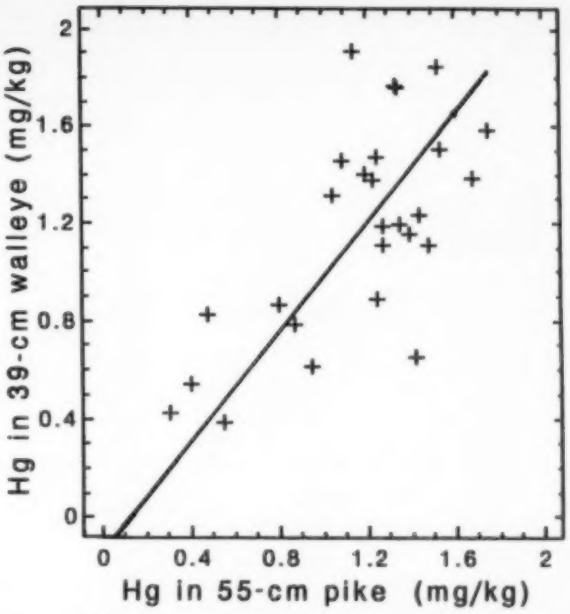

FIG. 3. Relationship (geometric mean regression) between mercury concentrations in walleye (39 cm) and northern pike (55 cm) for waters associated with the Churchill River diversion ($r^2 = 0.47$, $p < 0.001$).

transformations do assist with homogeneity of variance. An intercept of 0 falls within the 95% confidence limits for all equations (Table 4).

There is considerable overlap of data from the separate studies (Fig. 4). In general, fish collected in the 1970's from the Wabigoon/English River system had the highest mercury concentrations. Intermediate levels of mercury in northern pike and walleye generally represent the Churchill River reservoir development, while the lowest levels typically came from biota in undeveloped lakes in northern Minnesota.

We conducted an analysis of variance on BMPI (mercury in 39-cm walleye/mercury in 55-cm northern pike) values to determine if study area or mercury source had a significant bearing on the walleye to northern pike ratio. No significant effect was found ($p > 0.05$). Furthermore, there were no statistically significant correlations between the BMPI values and water quality or biophysical variables of Ontario lakes (Table 5).

Discussion

Factors Affecting Mercury Levels in Walleye and Northern Pike

From a quantitative modelling perspective, mercury sources and pathways to walleye and northern pike in nature are numerous, complex, (Fig. 5), and poorly understood for the vast majority of waters. It is generally recognized that most mercury in fish is in the form of methyl mercury and that most mercury in predators at the top of the food chain such as walleye and northern pike is derived from food and water. Methylation of mercury in the intestines of fish has been reported by Rudd et al. (1983), and methyl mercury in fish slime has been observed by Jernelov (1972); however, the net

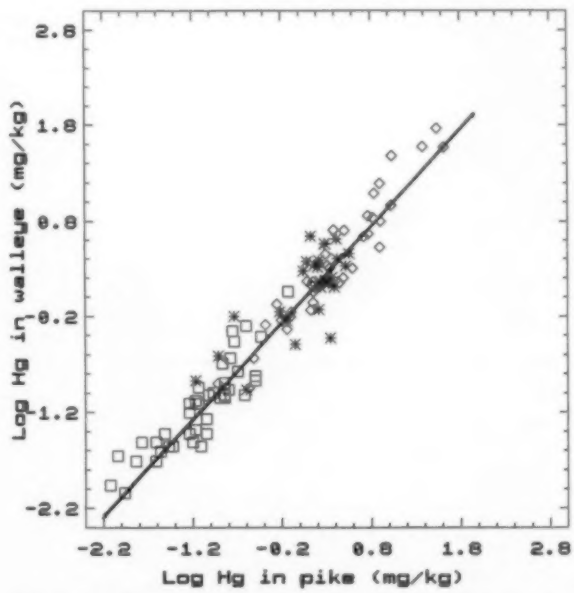

FIG. 4. Relationship (geometric mean regression) between log mercury concentrations in standardized walleye (39 cm) and northern pike (55 cm) for fish impacted by contaminated sediments (Wabigoon/English/Winnipeg River system) (diamonds), new reservoirs (Churchill River diversion) (asterisks), and atmospheric loadings (Minnesota lakes) (squares) ($r^2 = 0.93$, $p < 0.001$).

importance of these latter two factors to mercury concentrations in piscivorus predators has yet to be quantified.

TABLE 3. Mercury concentrations in walleye (39 cm) regressed against mercury concentrations in northern pike (55 cm) (geometric mean regression) for Clay, Ball, Separation, and Tetu lakes in the Wabigoon/English/Winnipeg River system. Samples collected (1970–85) during recovery of the system from point-source loadings in the 1960's. All regressions are significant at $p < 0.001$.

Lake		n	r^2	Slope	SER[a]	Intercept	Lower 95% confidence limit of intercept	Upper 95% confidence limit of intercept
Clay	log	10	0.78	1.02	0.19	0.14	−0.28	0.56
	Linear	10	0.79	1.15	0.66	−0.07	−1.3	1.4
Ball	log	9	0.86	0.94	0.14	−0.01	−0.24	0.044
	Linear	9	0.92	0.88	0.15	0.008	−0.33	0.34
Separation	log	9	0.92	1.01	0.10	0.02	−0.10	0.14
	Linear	9	0.90	0.95	0.18	0.10	−0.33	0.53
Tetu	log	11	0.92	0.85	0.14	−0.16	−0.26	−0.066
	Linear	11	0.95	0.63	0.09	0.20	0.064	0.33

[a]Standard error of the regression.

TABLE 4. Mercury concentrations in walleye (39 cm) regressed against mercury concentrations in northern pike (55 cm) (geometric mean regression) for fish affected by contaminated sediments (Wabigoon/English/Winnipeg River system), new reservoirs (Churchill River diversion), and atmospheric loadings (Minnesota). All regressions are significant at $p < 0.001$.

Sample set		n	r^2	Slope	SER[a]	Intercept
All collections	log	109	0.93	1.03	0.23	−0.012
	Linear	109	0.91	1.13	0.31	−0.12
Wabigoon/English/ Winnipeg River system	log	39	0.92	1.13	0.19	−0.07
	Linear	39	0.89	1.21	0.43	0.32
Churchill Diversion	log	27	0.58	1.00	0.29	0.002
	Linear	27	0.47	1.14	0.32	−0.16
Minnesota	log	42	0.80	1.02	0.21	−0.099
	Linear	42	0.77	1.17	0.09	−0.07

[a]Standard error of the regression.

TABLE 5. Correlations of log-standardized mercury in northern pike and walleye with lake variables for 79 lakes in Ontario where northern pike and walleye coexist. *Statistically significant, $p \leq 0.05$; **$p \leq 0.01$; ns, not significant. Biotic Mercury Partitioning Index (BMPI) = mercury in 39-cm walleye/mercury in 55-cm northern pike.

Lake variable	Northern pike[a]		Walleye[a]	BMPI	
	r	n	r	r	p
log	−0.21	76	−0.29**	−0.11	0.35
pH	−0.26*	62	−0.17	0.11	0.41
log alkalinity	−0.18	62	−0.05	0.17	0.17
log DOC	0.27*	54	0.27*	0.03	0.82
log Al	0.46**	54	0.47**	0.17	0.22
log Fe	0.48**	48	0.53**	0.21	0.15
Secchi	−0.22*	77	−0.33**	−0.17	0.14

[a]Data from Muncaster et al. (1989).

The relative importance of food and water pathways to mercury in walleye and northern pike populations requires substantial information to ascertain. More information on dietary contributions at different life stages of both species is needed, as well as a better understanding of the concentrations and speciation of mercury in waters and sediments. Knowledge of bioenergetic characteristics at the organism level is also required (Norstrom et al. 1976; Rodgers 1992).

Diet is believed to be the major pathway of mercury in both species (Phillips et al. 1980; Mathers and Johansen 1985; Grieb et al. 1990; Lindqvist et al. 1991a), and dietary differences between species can be marked (Mathers and Johansen 1985). Walleye generally have more mercury than northern pike at a given length (Phillips et al. 1980; Mathers and Johansen 1985) (Fig. 6) owing at least in part to higher metabolic rates (Mathers and Johansen 1985; Jackson 1991).

While the mercury body burdens in coexisting walleye and northern pike presented here can be traced to at least three separate sources — sediments contaminated by a point-source discharge, newly formed reservoirs, and the atmosphere — the significance of the several possible pathways from the source to fish remains unkown. For the Wabigoon River system, most mercury in fish has been attributed to the cycling of mercury from sediments contaminated with inorganic mercury (Armstrong and Hamilton 1973; Jackson et al. 1982; Rudd et al. 1983; Allan et al. 1984; Parks and Hamilton 1987). During preliminary studies most mercury in piscivorous fish

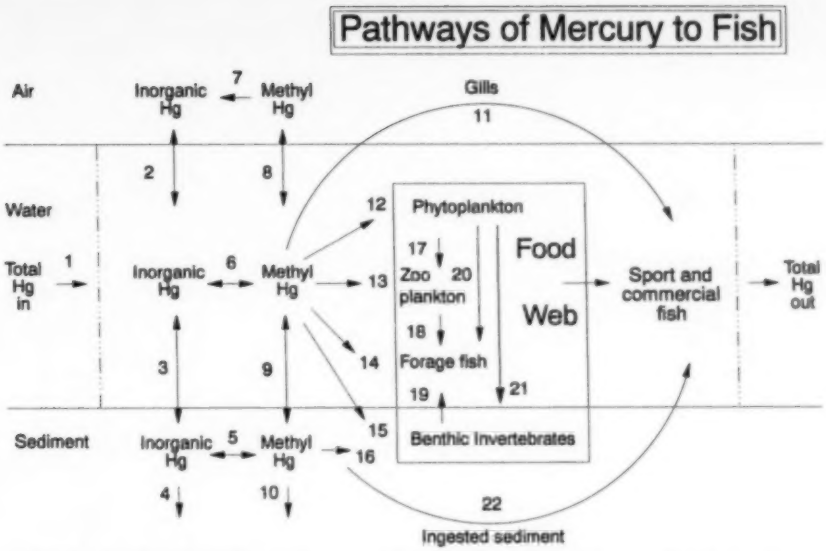

FIG. 5. Some pathways of mercury to top predators such as walleye and northern pike.

in this system was believed to be the result of processes 5, 9, and 11–21 (Fig. 5) whereby inorganic mercury was methylated in sediment and accumulated through the food web and across the gills. Rudd et al. (1980), however, demonstrated that methylation occurred in the water column. Parks et al. (1989) suggested that methylation in the water column (process 6) was of significance and suggested that this process was of particular importance to mercury accumulation in predators at the top of the food chain in this system.

Regardless of the pathway, mercury appears to be consistently partitioned between these two species of fish on a site-specific basis over time at three of the four sites (Fig. 1). Why northern pike accumulate more mercury at a given size in comparison with walleye with distance downstream of the historical point source is unknown, but on the basis of previous discussions, differences in diet are hypothesized. The high correlations of linear regressions to describe these site-specific standardized fish relationships were surprising. There are many nonlinear functions governing the net accumulation of mercury in each species, and a wide range of half-lives for mercury in biota recovering from mercury contamination can be expected (Fagerström et al. 1974, 1975; Norstrom et al. 1976). These results, however, suggest that linear approaches to establishing these relationships may be appropriate.

The effects of mercury contamination on whole populations of northern pike and walleye were examined by regressing the slopes of the log mercury concentration versus log length regression (Table 1b) against time as the watercourse recovered from historical discharges. There was only one significant relationship, for northern pike at Tetu Lake, where the contaminated English River joins the Winnipeg River (r = 0.66, p = 0.05). This relationship suggests that the accumulation rates in northern pike in Tetu Lake have changed with time. A detailed review of fish collection methodology, however, suggests that the relationship may be the result of sampling errors. Northern pike and walleye from Tetu Lake were pooled from several sites (P. MacDonald, OMNR, personal communication), some of which were more

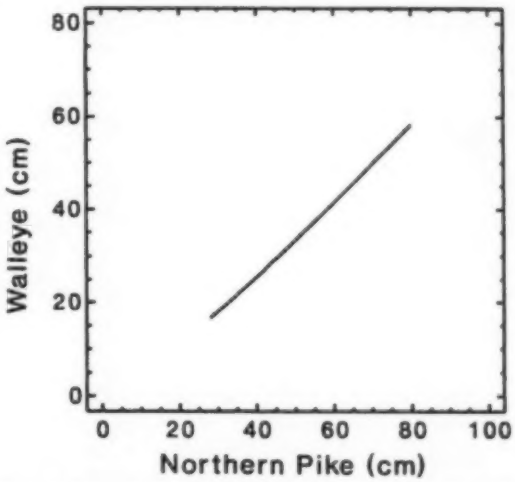

FIG. 6. Relationship between the length of walleye and northern pike where mercury concentrations are equal, for 79 Ontario lakes.

influenced by contaminated English River flows than others (Parks et al. 1991b). Because the fish captured were not identified by lake site, the effects of site-specific contamination becomes a confounding variable when mercury–length relationships are analysed. As sampling the various lake sites over time has not been random (P. MacDonald, OMNR, personal communication), sampling bias may well have been introduced. The effects of such bias would be more pronounced in northern pike collections than in walleye collections because walleye are more wide ranging and less affected by localized mercury sources (Jackson 1991). The suggested difference in accumulation rates for northern pike in Tetu Lake could explain why the intercept for the GMR regression for the standardized walleye – northern pike relationship (Fig. 1d) is significantly different from 0 at the

TABLE 6. Geometric mean regression for modelled concentrations in 39-cm walleye versus observed values.

Geographical area/issue	n	r^2	Slope	Upper 95% confidence limit of slope	Lower 95% confidence limit of slope	Intercept	Upper 95% confidence limit of intercept	Lower 95% confidence limit of intercept
79 Ontario lakes	79	0.64[a]	1.03	1.17	0.89	−0.1	−0.002	−0.20
Lake St. Clair (historical loadings)	12	0.88[a]	0.96	1.20	0.73	0.02	0.12	−0.08
Reservoirs								
La Grande 2	5	0.96[b]	1.35	1.87	0.84	−0.47	−0.076	−0.87
Boyd	4	0.97[b]	1.21	1.79	0.64	−0.33	0.073	−0.73
Cedar	3	0.94[c]	0.66	2.45	−1.14	0.18	2.2	−2.6

[a] $p < 0.001$.
[b] $p < 0.05$.
[c] $0.2 > p < 0.1$.

95% confidence level. For other sites, Clay, Ball, and Separation lakes, mercury concentrations in various sizes of northern pike and walleye are assumed to have responded similarly to those of the standard fish.

Elevated mercury concentrations in fish in the Churchill River diversion have been attributed to mercury methylated in newly submerged terrestrial organic matter (process 5) (Bodaly et al. 1984, 1987; Hecky et al. 1987a, 1987b, 1987c; Jackson 1987, 1988; Ramlal et al. 1987; Ramsey and Ramlal 1987a, 1987b; Rannie and Punter 1987). For these waters the pathways of mercury to walleye and northern pike (and whitefish, perch, and shiners) also appear to be species specific (Jackson 1991). The aberrantly low r^2 for the walleye – northern pike relationship here (0.58) in comparison with other groups of lakes (Table 4) may be attributed to differences in species habitat preferences and spatial heterogeniety in methyl mercury production in flooded lakes (Jackson 1991).

The atmosphere is implicated as a major source of mercury in walleye and northern pike in northern Minnesota (process 2) (Sorensen et al. 1990). Nearly all mercury from the atmosphere is in the inorganic form (Bloom 1989), and the relative importance of several methylation and accumulation processes as shown in Fig. 5 remains to be elucidated (Sorensen et al. 1990).

Dispite the uncertainty about methylation processes and pathways of mercury to walleye and northern pike in these studies on chlor-alkalai impacted waters, reservoirs, and pristine lakes, the regression in Fig. 6 clearly suggests that mercury is partitioned between these two species regardless of mercury source.

No species-specific differences in environmental factors affecting mercury concentrations in walleye or northern pike in coexisting populations were identified for Ontario waters using the BMPI (Table 5). These results are perhaps not too surprising given the high r^2 in Fig. 6, but the results do suggest that the species differences reported by Wren et al. (1991) for pH, alkalinity, and area for 79 lakes in Ontario with coexisting walleye and northern pike populations may not be particularly significant, as the correlations between fish and environmental factors presented here for Ontario lakes are identical or very similar to those presented by Wren et al. (1991).

Modelling Implications

Our observation that there are no species differences may have useful modelling implications. It may, for instance, be possible to model mercury levels in coexisting populations of walleye and northern pike, with information only on one or the other species. Additional data on common environmental variables would not necessarily increase the accuracy with which the partitioning of mercury between these species could be predicted.

Currently, most of the models used to predict mercury levels in a given species of fish from water chemistry and other environmental variables are empirical and usually have only modest r^2 values (although small localized watersheds can be an exception; Spry and Weiner 1991), leaving a significant proportion of the variance unexplained (Lathrop et al. 1991). Moreover, these models are commonly of limited utility on waters outside the geographic range where they were developed, probably because the model itself is dependent upon the distribution of bioaccumulation variables from the particular lakes on which the model was derived. As an example, the models developed by Håkanson (1980) and Håkanson et al. (1988) for Swedish lakes were not very satisfactory when applied to Finnish waters (Verta 1990). Lindqvist et al. (1991a) provided some good examples of the use and misuse of such models.

Our analysis suggests that in many cases, predicting mercury concentrations in a particular species and population can be achieved more accurately with interspecies relationships than through modelling with the environmental variables used in much of the literature on the subject. We suggest that the BMPI may be useful in modelling at the community level — at the top of the food chain at least — by providing a framework with which to determine which organisms are affected by the same environmental variables.

Model Testing

Site-specific relationships

From our analysis of fish in the Wabigoon/English/Winnipeg River system, we expect linear relationships to exist between mercury concentrations in standardized northern pike and walleye in similar ecosystems where only mercury loadings are different. We tested this hypothesis using data on another watercourse where mercury loadings changed substantially (decreased): Lake St. Clair on the Saint Lawrence River (Neary 1981). The average BMPI (39-cm walleye/55-cm northern pike) value for all waters tested was used initially:

(1) 39-cm walleye = $k \times$ 55-cm northern pike

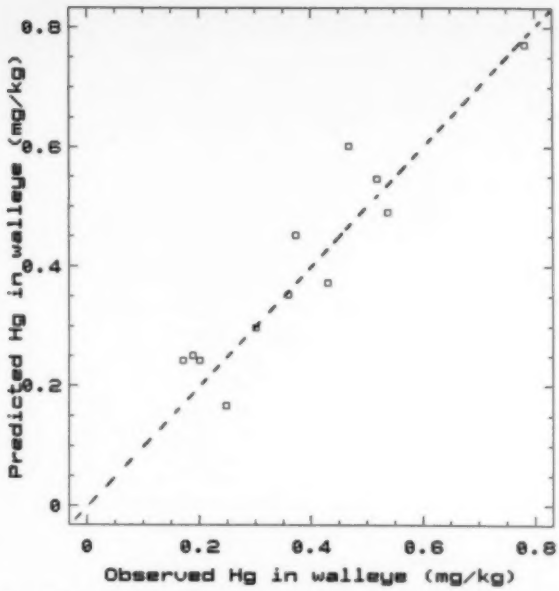

FIG. 7. Predicted versus observed mercury concentrations for walleye (39 cm) in Lake St. Clair, Ontario ($r^2 = 0.88$, $p < 0.001$). Broken line: $y = x$.

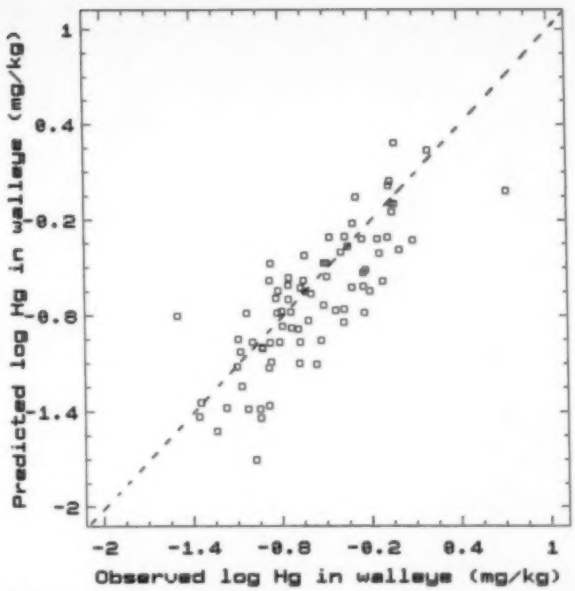

FIG. 8. Predicted versus observed concentrations in walleye (39 cm) for the Ontario Sportfish Data Base ($r^2 = 0.64$, $p < 0.001$). Broken line: $y = x$.

where k = average BMPI = 1.0 ± 0.23. There is a high correlation between predicted and observed mercury values in walleye ($r^2 = 0.88$); however, the slope of 0.55 differed significantly from 1 ($p > 0.05$). This difference is the result of the actual BMPI value for Lake St. Clair being much less than 1. From results of multiple samplings for this system the mean BMPI for Lake St. Clair is 0.53 ± 0.12 ($n = 12$). Improved predictive capabilities can be obtained by using the site-specific BMPI value. With an BMPI value of 0.53 for Lake St. Clair, the 95% confidence limits for the slope for the regression between predicted and observed values were not significantly different from 1 and the 95% confidence limits for the intercept were not significantly different from 0 (Fig. 7; Table 6).

Multisite relationships

We suggest that the GMR regression incorporating all data could be useful as a generic equation for estimating mercury in other walleye and northern pike populations where there is little or no information available on the mercury source or pathways for either species. We show here that mercury concentrations in standardized northern pike and walleye are highly correlated regardless of (1) mercury source (atmospheric, historically contaminated sediments, new reservoirs), (2) degree of contamination (Clay, Ball, Separation, and Tetu lakes), (3) changing mercury loadings to the watercourse (Wabigoon/English/Winnipeg River system, and (4) changes in water quality. As the relationship covers a very wide range of concentrations, the results from fish collected from other waters can be expected to fall within the range of the model. The model should be most applicable to other shield waters, as it was derived from a data base dominated by shield lakes.

The predictive capability of the regression in Table 4 and Fig. 4 was tested on 79 lakes from the Ontario Sportfish Data Base (Wren et al. 1991) (Fig. 8). This data base is for lakes that receive mercury from both point and diffuse sources, including impounded lakes. Although the predicted values were on average 9% lower than those observed, an r^2 of 0.64 between predicted and observed values suggests that the equation has some merit, particularly as the r^2 for this relationship is slightly higher than that for the walleye – northern pike relationship reported by Wren et al. (1991) who used slightly different sized fish and log–linear relationships to standardize for fish length. The slope of the relationship between predicted and observed values was not significantly different from 1 at the 95% confidence limits nor was the intercept significantly different from 0 ($p > 0.05$) (Table 6).

The predictive capability of the generic equation was also tested on reservoirs. The correlations between predicted and observed mercury levels for three other reservoirs (Johnston et al. 1991) are significant (Fig. 9; Table 6). The 95% confidence limits for the slope of the GMR regression did not differ significantly from 1, but the 95% confidence limits for the intercept were significantly different in one case (Table 6).

Biomonitoring

Many of the proposed models for mercury in walleye and northern pike populations are believed to be capable of improving the efficiency of biomonitoring programs. Clearly, the geometric mean regressions for Clay, Ball, Separation (Fig. 1), and St. Clair lakes in conjunction with site-specific population characteristics for mercury accumulation could be used to reduce monitoring requirements for either species. Currently, it is not possible to provide adequate public health advisories for all waters where some northern pike or walleye may have levels that exceed health guidelines. The

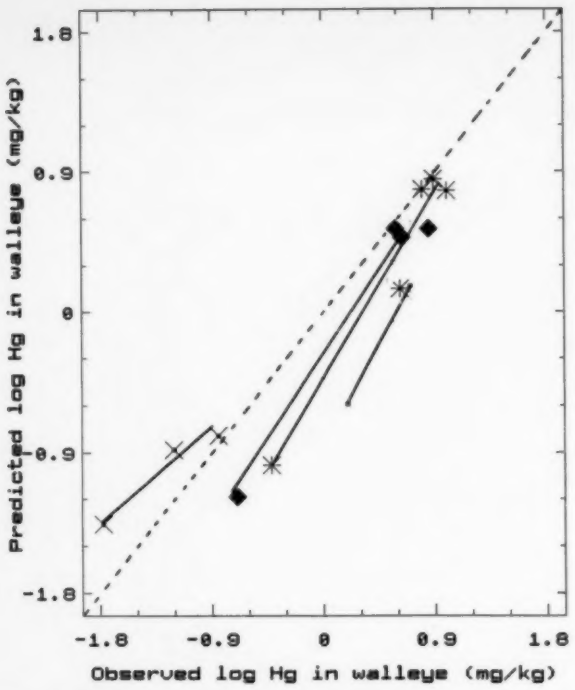

FIG. 9. Predicted versus observed concentrations in log walleye (39 cm) for three reservoirs: La Grande 2 (asterisks) ($r^2 = 0.96$, $p < 0.05$), Boyd (diamonds) ($r^2 = 0.97$, $p < 0.05$), and Cedar (crosses) ($r^2 = 0.94$, $0.1 > p > 0.2$). Broken line: $y = x$.

relationships presented here may be useful in reducing sampling requirements.

There is evidence to suggest that many other "generic" relationships could be developed between standardized organisms collected from a common habitat (MacCrimmon et al. 1983; Parks 1988; Cope 1990; Sorensen et al. 1990). The strength of the correlations between other biota may or may not be as high as that observed for the walleye and northern pike, depending upon the degree to which both organisms share the same habitat, food web, and history of exposure. For example, good empirical interrelationships are anticipated between members of the perch family (yellow perch (*Perca flavescens*), sauger (*Stizostedion canadense*), and walleye) and the pike family (*Esox masquinongy*, *E. niger*, *E. americanus*, and *E. vermiculatus*). Interspecies relationships between cool-water species or cold-water species may have higher r^2 values than relationships between cool- and cold-water species due to the differences in habitat and food webs.

We suggest that developing these relationships would be useful, given the major difficulties in identifying and quantifying the importance of various pathways and pharmocokinetics for any given species. Indeed the species-specific information requirements for modelling mercury body burdens are very large (cf. Norstrom et al. 1976), and given the difficulties in ascertaining food web vectors for mercury, the models are, for most intents and purposes, not amenable for routine site-specific application.

The development of multispecies biotic relationships, as proposed here, offers four distinct advantages in modelling mercury contamination.

(1) There are more laboratories that can adequately analyse for mercury in biota than for other key compartments such as water. Consequently, there are large quantities of biological data that could be used to confirm, clarify, and extend the observations presented here. Because we are using relationships between species, concerns about laboratory quality assurance are minimized; as long as both species of fish are analysed at the same laboratory for any given data point, analytical errors would be off-setting.

(2) Measurements of correlates for bioavailable mercury (various mercury species) in water and sediments are, and will continue to be, problematic owing to analytical limitations and a lack of understanding of exactly what is bioavailable.

(3) Quantitative information on diet and water contributions to body burdens is not required to determine mercury concentrations in a given species.

(4) The relationships between mercury in walleye and northern pike in the Wabigoon/English/Winnipeg River system reflect the response of the aquatic community to decreased mercury loadings, both external and internal, assuming that metabolic factors and food webs remained the same during the study period. Therefore, these loadings can be coupled with loading data to infer cause and effect.

Development of biological relationships between geographically diverse but overlapping species could provide a rationale to select bioindicators suitable for national or international purposes for those countries where the geographical range of any single species is inadequate. Perhaps most useful would be the selection of a standard species with wide geographic distribution which can be found in a wide range of aquatic habitats including both cold and warm water. Northern pike, walleye, or bass may be suitable. Relationships between one indicator species and other organisms could possibly be utilized to provide common "standards of exposure" for other organisms.

Finally, we note that there are other contaminants such as certain trace organics that also increase with fish size. While different factors govern the bioaccumulation of these contaminants, we hypothesize that many of the concepts applied to evaluating the partitioning of mercury in the aquatic community as discussed here could be applied to other contaminants of concern such as dioxins, PCBs, Mirex, and DDT.

Conclusions

Highly significant relationships exist between mercury levels in coexisting walleye and northern pike regardless of whether mercury body burdens resulted from atmospheric loadings, new reservoir development, or contaminated sediments. These relationships also exist where mercury levels in the aquatic environment changed rapidly as a result of decreased external loadings.

There were no differences in the partitioning of mercury between species that could be inferred from water pH, alkalinity, dissolved organic carbon, conductivity, calcium, magnesium, and iron, all of which have been identified as being important to the bioaccumulation of mercury in one or both species. The relationships presented may be useful in modelling studies and biomonitoring programs.

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Phosphorus Metabolism Monitored by ^{31}P NMR in Juvenile Sea Scallop (*Placopecten magellanicus*) Overwintering in Pearl Nets at a Nova Scotian Aquaculture Site[1]

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^{31}P Nuclear magnetic resonance (NMR) spectroscopy has been used to examine in vivo phosphorus metabolism in juvenile sea scallop (*Placopecten magellanicus*) from an aquaculture site in Nova Scotia. NMR-visible arginine phosphate (Arg P) concentrations declined from summer to winter whereas inorganic phosphate (P_i) levels increased and those of adenosine triphosphate (ATP) remained relatively constant. Our results indicate that, in the scallop that uses the phosphagen Arg P to replenish ATP, the ratio of Arg P to P_i is a better indicator of energetic status than is the ratio of adenylate concentrations known as adenylate energy charge. Scallops collected in fall and winter, when exposed to hypoxic conditions, showed pronounced decreases in Arg P associated with increases in P_i and decreases in ATP levels whereas these remained relatively unchanged in scallops collected in summer. Scallops maintained under simulated winter conditions showed lower levels of Arg P associated with poor nutrition, and a response to hypoxia similar to field scallops. The marked seasonality in concentrations of phosphorus metabolites emphasizes their potential importance as useful biomarkers in environmental stress assessment and the optimization of hatchery and grow-out conditions.

On a utilisé la spectroscopie de résonnance magnétique nucléaire (RMN) du ^{31}P pour examiner in vivo le métabolisme du phosphore chez des pétoncles géants (*Placopecten magellanicus*) juvéniles provenant d'un site d'aquaculture de la Nouvelle-Écosse. Les concentrations de phosphate d'arginine (Arg P) visibles par RNM ont diminué de l'été à l'hiver, alors que les concentrations de phosphate inorganique (P_i) ont augmenté et que celles d'adénosine-triphosphate (ATP) sont demeurées relativement constantes. Nos résultats indiquent que, chez les pétoncles qui utilisent l'Arg P phosphagène pour renouveler leur ATP, le rapport Arg P/P_i est un meilleur indicateur de l'état énergétique que le rapport des concentrations d'adénylate appelé charge énergétique adénylique. Les pétoncles prélevés en automne et en hiver, quand ils sont exposés à des conditions hypoxiques, présentent des diminutions prononcées de l'Arg P associé aux augmentations de P_i et des diminutions des concentrations d'ATP, alors que ces valeurs restent relativement inchangées chez les pétoncles recueillis en été. Les pétoncles maintenus dans des conditions d'hiver simulé présentaient de plus faibles concentrations d'Arg P associées à une mauvaise nutrition, ainsi qu'une réponse à l'hypoxie semblable à celle des pétoncles prélevés sur le terrain. Le caractère saisonnier marqué des concentrations de métabolites du phosphore souligne leur importance possible comme biomarqueurs utiles pour l'évaluation du stress environnemental, ainsi que pour l'optimisation des conditions des écloseries et des bassins d'affinage.

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Increasing interest and effort in the development of a commercially viable scallop aquaculture industry in eastern Canada prompted this investigation of seasonal variation in the energetic state of juvenile scallops. Poor survival of juveniles under "grow-out" conditions in the field is an important limitation in aquaculture, and thorough knowledge of physiological and ecological factors that affect juveniles is therefore critical to optimization of hatchery and grow-out conditions, as well as to an understanding of the dynamics of whole populations.

Growth rates of scallops are greatly reduced during winter (e.g., Sundet and Vahl 1981; Bricelj et al. 1987), and seasonal changes in energy reserves have been documented and related to reproductive output in the adult scallops *Placopecten magellanicus* (Thompson and MacDonald 1989), *Argopecten irradians irradians* (Epp et al. 1988), and *Chlamys islandica* (Sundet and Vahl 1981), but little is known about such changes in the energetics of juveniles. Scallops, unlike most other bivalve molluscs, can swim, replenishing ATP via anaerobic metabolic pathways (de Zwaan et al. 1980). The compounds that maintain energy production during vigorous swimming (functional anaerobiosis) are essentially the

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same as those metabolized during environmentally induced anaerobiosis (Gäde 1983). The concentrations of adenylates, particularly ATP, in relation to their hydrolysis products have been used as indicators of energetic state (Ivanovici and Weibe 1981; Nicchitta and Ellington 1983). In organisms such as the scallop, which have a phosphagen system (Arg P) to replenish ATP levels, the phosphagen is present in higher concentration than the adenylates and may prove to be a more useful indicator of energetic status than the concentrations of adenylates.

We have used ^{31}P nuclear magnetic resonance (NMR) in vivo to measure the concentrations of phosphorus-containing metabolites in intact juveniles of the sea scallop *Placopecten magellanicus*. This spectroscopic technique detects signals from intracellular phosphorus metabolites that are not constrained in freedom of movement by binding to macromolecules or incorporation in membranes. Monitoring of phosphorus metabolite concentrations by ^{31}P NMR is well established with organisms from a wide range of phyla and has proven to be immensely powerful in obtaining reliable information in vivo on the definition of, and interaction between, biosynthetic pathways involved in bioenergetic processes. Some previous applications to molluscs have been reported, including experiments with excised muscle tissue (Burt et al. 1976; Barrow et al. 1980; Ellington 1983a, 1983b; Graham et al. 1986) and studies in vivo with intact animals of the effects of parasite invasion in the snail *Biomphalaria glabrata* (Thompson and Lee 1985), salinity stress on abalone (*Haliotis* sp.) (Higashi et al. 1989), and hypoxia in *Haliotis* (Tjeerdema et al. 1991) and the mussel *Mytilus edulis* (Fan et al. 1991).

This study examines the effects of stresses imposed by overwintering in pearl nets on the energetic state of juvenile sea scallop by comparing the concentrations of NMR-visible phosphorus metabolites in juveniles harvested in summer with those in others collected in fall and winter. A parallel study was performed on juvenile scallops held in aquaria under conditions of controlled temperature and nutrition. The extent to which the concentrations of phosphorus metabolites, as perturbed by experimentally induced hypoxia, might reflect seasonally induced changes in the aerobic and anaerobic metabolic capacity was also examined.

Materials and Methods

Scallops of shell height 13–16 mm hatched at the Fisheries Resource Development Ltd. operation at the Sandy Cove Aquaculture Research Station of the National Research Council's Institute for Marine Biosciences, Halifax, Nova Scotia, and grown in pearl nets suspended below the thermocline at a depth of approximately 12 m at Indian Point in Mahone Bay, Nova Scotia, were used in this study. Collection dates ranged from June 1991 to May 1992. Animals were transported from the field in seawater to a flowing seawater aquarium system maintained at seasonally adjusted seawater temperatures and fed a mixed algal diet for approximately 24 h prior to NMR measurements. The NMR spectra showed the effects of season on the concentration of phosphorus metabolites. These "field" data were divided into three categories, according to the collection time: (1) June through to October, when water temperature fluctuated in the range 18–12°C and food was abundant ("summer"), (2) November to December, when water temperatures decreased to 4°C and phytoplankton numbers were declining ("fall"), and (3) early to mid-April, prior to the spring bloom, when water temperatures ranged from −2 to 4°C and phytoplankton densities were minimal ("winter").

As temperature and nutritional levels are major seasonal variants, their effects on phosphorus metabolism of juvenile scallops from the same location in Mahone Bay were examined in a laboratory-controlled 2 × 2 factorial experiment using a seawater supply from the Northwest Arm of Halifax Harbour. The water temperature was either held at approximately 15°C (the approximate mean of summer seawater temperatures in Mahone Bay) or was allowed to follow the ambient temperature of the seawater pumped from the Northwest Arm, which was essentially the same as the ambient temperature at Indian Point. The organisms were either left unfed or were continuously fed on a mixture of cultured algae (*Isochrysis galbana*, *Thalassiosira pseudonana*, and *Chaetoceros muelleri*) at a mean cell concentration of approximately 5×10^3 cells·mL^{-1}. Each combination of temperature/feeding was assigned at random among three aquaria, each of which contained approximately 75 scallops. After an initial 2-wk equilibration period at 11°C during which all scallops were fed, they were kept in the various treatment conditions for 6 wk prior to NMR analyses. The seawater supply was filtered successively through sand, 5-μm cellulose, and 1-μm polypropylene filters (Atlantic Purification, Dartmouth, Nova Scotia) at a flow rate of approximately 300 mL·min^{-1}. The filters were changed every other day.

Maintenance of Scallops during NMR Measurements

Groups of 25–30 scallops were placed in a 20-mm (outside diameter) glass NMR tube (Fig. 1), equipped for perfusion with aerated artificial seawater medium (McLachlan 1973) in which the phosphate concentration was 20 μM. This medium was pumped to the bottom of the NMR tube at 12 mL·min^{-1} and displaced through an outflow tube. Temperature in the NMR tube was controlled by the flow-through of perfusate solution pumped from an external temperature-controlled reservoir of medium. The medium was made hypoxic when required by bubbling with nitrogen. An Ingold oxygen monitoring system (model no. IL531) with a detection limit of 10 ppb O_2 was used to measure levels of dissolved oxygen in the perfusate solution leaving the NMR tube and in the reservoir. Scallops were returned to the aquarium system after NMR examination and observed for a week.

Acquisition of Spectra

NMR spectra were recorded with a Bruker MSL 300 spectrometer, operating at 121.5 MHz. The magnetic field was shimmed before each series of runs using a solution of 50 mM Na_2HPO_4 in 10% D_2O/90% H_2O in a 20-mm (outside diameter) tube identical to that used to contain the scallops. This solution was also used as a sensitivity standard, and receiver gain was kept constant. The probe was matched and tuned for each sample individually. Spectra were collected with a 90° pulse, 16 384 time domain points, and a sweep width of 16 129 Hz. Peak areas in NMR spectra are proportional to metabolite concentrations, provided that the intervals between pulses are long compared with the spin lattice relaxation times (T_1) so that saturation is avoided.

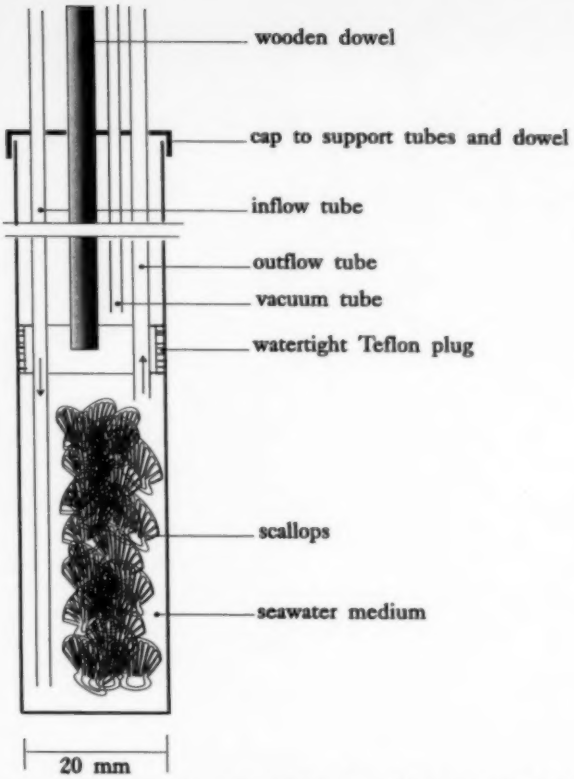

FIG. 1. Diagram of juvenile scallops in a 20-mm (outside diameter) NMR tube with flowing artificial seawater. The vacuum tube above the Teflon plug removed any medium escaping around the plug.

The T_1 values (in seconds) used in this study were obtained from adult scallop muscle tissue: P_i 3.3, Arg P 1.6, and ATP 0.4, 0.4, and 0.3 (α, β, and γ phosphate residues, respectively). These values are similar to those reported for other molluscs (Barrow et al. 1980; Ellington 1983b; Schanck et al. 1986). Our spectra were routinely acquired with a recycle delay of 3 s. A longer recycle delay time was not used because the time allowable for signal acquisition from living tissues is limited by inherently low signal to noise ratios and the innate problem in NMR in vivo measurements of maintaining physiologically appropriate steady-state conditions in the NMR tube during prolonged periods of data acquisition. However, the P_i resonance was not fully relaxed at the 3-s recycle delay time. Therefore the peak area for P_i was corrected by using the ratio of the area of the P_i peak in a spectrum of adult scallop adductor muscle tissue, acquired under conditions of full relaxation (30-s recycle delay), to its area at a 3-s recycle delay, 2.20 ± 0.03. This saturation factor was assumed to be constant in all the experiments, and the area of the P_i peak was corrected accordingly.

The free induction decays acquired from the live material were multiplied by an exponential factor corresponding to a line broadening of either 10 Hz (in the case of juvenile scallops) or 2 Hz (excised muscle tissue) prior to Fourier transformation. Proton decoupling was not used, to avoid unquantifiable intensity changes due to nuclear Overhauser

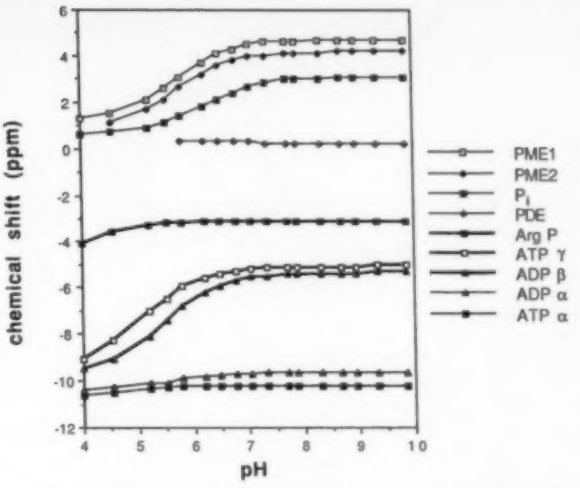

FIG. 2. ^{31}P chemical shifts of phosphate compounds versus pH in a PCA extract of scallop muscle. Compounds: phosphomonoesters (PME1, PME2), inorganic phosphate (P_i), phosphodiesters (PDE), arginine phosphate (Arg P), ATP (resonances of α and γ ^{31}P), and ADP (resonances of α and β ^{31}P). The chemical shift of the P_i resonance was used to determine intracellular pH from spectra recorded in vivo.

enhancements. Spectral line widths usually exceeded both the field inhomogeneity and the ^{31}P-^{1}H coupling and were not further reduced by decoupling. Orthophosphoric acid at 85% concentration was used as an external reference for 0 ppm. Resonances were integrated on a constant scale after baseline correction using a low-order polynomial such that regions between peaks produced zero integral.

To assist in peak assignment and quantitation, NMR spectra were also obtained from the excised adductor muscles of adult scallops (10–13 cm shell height) collected from natural populations in Mahone Bay. The excised tissue was maintained in the NMR tube in a similar manner to live juveniles. These in vivo spectra were compared with in vitro spectra from muscle extracts prepared by a method adapted from Barrow et al. (1980). For the latter, the tissue was frozen with liquid nitrogen, weighed, and powdered in a mortar cooled with liquid nitrogen and then homogenized with ice-cold 7% perchloric acid (PCA) (about 10 mL·g tissue^{-1}) in a Potter-Elvehjem homogenizer and centrifuged at 3000 × g for 5 min. The pellet was reextracted with 5 mL of 7% PCA·g tissue^{-1}. The combined supernatants were neutralized with K_2CO_3 and allowed to stand for 30 min on ice. The precipitated potassium perchlorate was removed by centrifugation at 3000 × g for 5 min and the supernatant was lyophilized. This material was redissolved in 10% D_2O/10 mM EDTA in 10-mm (outside diameter) NMR sample tubes.

Peak Assignment and Quantitation

The ^{31}P chemical shifts of metabolite resonances are affected by both pH and ambient ionic composition (Roberts and Jardetzky 1981); hence the relationships between pH and chemical shifts were defined in the ionic environment provided by cell extracts (Fig. 2). ^{31}P spectra of lyophilized PCA extracts (see above) from adult scallop muscle were

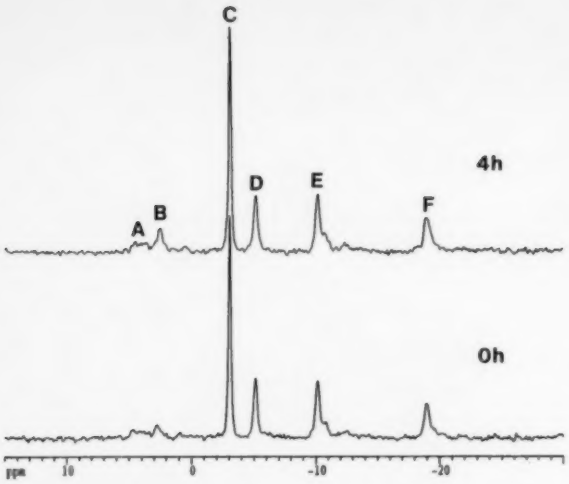

FIG. 3. ^{31}P NMR spectra in vivo of juvenile scallops collected in July. Lower trace, 0 h: before hypoxic conditions applied; upper trace, 4 h: after 4 h of hypoxia. Resonance assignments: A, phosphomonoesters; B, P_i; C, Arg P; D, ATP γ/ADP β; E, ATP α/ADP α/NADH/NADPH; F, ATP β.

assigned by comparison with published values (Barrow et al. 1980; Ellington 1983a, 1983b; Thompson and Lee 1985; Higashi et al. 1989), chemical shift changes with pH (Fig. 2), and the superposition of unknown peaks with those of added authentic compounds. As the chemical shift of Arg P (Fig. 2) does not vary within the physiological pH range, it was used as an internal reference. Corresponding peaks were readily identifiable, although considerably broader, in the spectra of living scallops, which closely resembled those of abalone foot muscle (Higashi et al. 1989) and mussel posterior adductor muscle (Fan et al. 1991). Areas of peaks from living samples were standardized, with allowance for saturation factors and number of scans, against that of the resonance from 50 mM Na_2HPO_4 obtained under the same conditions in an identical tube. To assist in quantitation, spin-lattice relaxation times (T_1) were measured without ^1H decoupling, using the standard Bruker MSL pulse sequence for the inversion recovery method and a delay time of 30 s. Concentrations of metabolites were calculated in relation to the weights of wet and dry tissue determined, following each NMR run, by blotting, removing the body tissue, weighing, lyophilizing, and reweighing. It was assumed that the 25–30 scallops were uniformly distributed over a volume greater than the sensitive volume of the probe and that the sensitive volume was the same when occupied by the standard solution. Effects of differential probe damping between the scallop samples and the standard solution were minimized by separately tuning and matching the probe response for each sample and monitoring the bandwidth of the probe response with a sweep oscillator. All statistical analyses of NMR data were performed using Systat 5.1.

Adenylate Energy Charge

The resonance at −18.7 ppm in the ^{31}P NMR spectra arises solely from ATP (β phosphate), while the resonance at −5.0 ppm (γ phosphate of ATP) overlaps the resonance from the β phosphate of ADP (Fig. 3). Thus the ratio of these resonances is the ratio ATP:(ATP + ADP). The indicator of potential energy usually measured by in vitro assay is the adenylate energy charge (AEC) (Atkinson and Chapman 1979), which can be calculated from the ratio ATP:(ATP + ADP) by assuming that the adenylate kinase reaction is at equilibrium (Steen 1986).

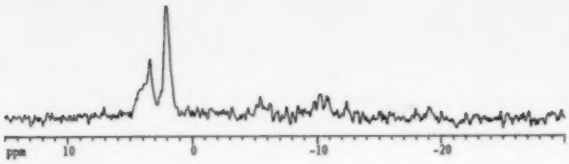

FIG. 4. ^{31}P spectrum of body tissues (excluding the adductor muscle, and composed mainly of digestive diverticulae) excised from juvenile scallops. These signals differ in chemical shift from that of P_i in intact juveniles (see Fig. 3) and correspond to those of digestive diverticulae dissected from adult scallops.

Results

^{31}P spectra of juvenile scallops in vivo (Fig. 3) closely resemble those from abalone (Higashi et al. 1989) and mussels (Fan et al. 1991), showing, for example, the resonances at −3 ppm (Arg P), −18.7 ppm (ATP β), and −5 ppm (ATP γ and ADP β). The inorganic phosphate resonance occurs in the vicinity of 2.7 ppm, its precise location indicating intracellular pH.

To distinguish the NMR signals arising from juvenile adductor muscle from those of the other body tissues (e.g., digestive diverticulae, gills, and mantle), a spectrum was acquired after removal of the adductor (Fig. 4).

The two major peaks evident in the juvenile body tissues, excluding the adductor, corresponded to those observed in the spectrum of digestive diverticulae dissected from adult scallops. The chemical shifts of these two signals (3.35 and 2.04 ppm) were distinct from the P_i peak of intact juveniles (2.70 ppm) and, as they were not apparent as major peaks in the spectrum of the entire juvenile animal, it was concluded that the remaining detectable phosphorus signals in Fig. 3 were from the adductor. The spectrum of perfused adult muscle tissue resembled that of intact juveniles (Fig. 3). It should be noted that the ^{31}P spectrum of the PCA extract of adult muscle tissue was also similar to the spectrum in vivo except for the presence of a small peak in the phosphodiester region and greater resolution of resonances in the phosphomonoester region of the extract.

The effect of season on phosphorus metabolites in juvenile scallops was established from scallops collected from the field during summer, fall, and winter periods (see Materials and Methods). As winter seawater temperatures in the field were lower than those in the NMR tube by as much as 8°C, the effect of a sudden increase in temperature during NMR measurements was examined using juvenile scallops collected in December. The temperature of the medium pumped into the NMR tube was raised to 20°C for 3 h, after an initial acquisition at 10°C, and then returned to 10°C for 1.5 h. No detectable changes occurred in the ^{31}P NMR spectra in the winter-acclimated scallops.

The proportion of Arg P (as a percentage of the summed areas of P_i, Arg P, ATP, and ADP resonances) declined from a summer high of 63% to a winter low of 17% ($P \leq 0.001$),

TABLE 1. P_i, Arg P, and ATP in juvenile scallops harvested from Indian Point, Mahone Bay, N.S., at various seasons, expressed as percentages of the total of NMR-visible P_i, Arg P, ATP, and ADP (means ± 1 SD) and as μmol·g dry wt^{-1} (in parentheses).

Season	P_i	Arg P	ATP	n^a
Summer (June–Oct.)	11 ± 5	63 ± 5	22 ± 5	6
	(22 ± 12)	(176 ± 74)	(52 ± 20)	4
Fall (Nov.–Dec.)	20 ± 6	54 ± 5	20 ± 3	4
	(57 ± 29)	(148 ± 18)	(45 ± 5)	2
Winter (early to mid-Apr.)	70 ± 11	17 ± 10	11 ± 2	4
	(106 ± 14)	(63 ± 49)	(22 ± 14)	2

[a]Due to sample loss in workup, concentration values in parentheses represent the mean of only four groups of summer animals, two groups of fall animals, and two groups of winter animals.

and P_i increased concurrently ($P \leq 0.001$) from a summer low of 11% to a winter high of 70% (Table 1; Fig. 5). For ATP, an independent *t*-test revealed no difference between summer and fall samples ($P = 0.3$) (mean value 21 ± 4%), but the level in the winter sample was significantly lower (11 ± 1.5%)($P = 0.002$). The adenylate ratio ATP:(ATP + ADP), 0.82 ± 0.07, did not vary significantly with season ($P = 0.7$).

The same trends in the phosphorus metabolite composition were evident between seasonal groups (Table 1) with a mean value for Arg P ranging from a high of 176 μmol·g dry wt^{-1} in summer to 63 μmol·g dry wt^{-1} in winter. For (ATP + ADP), a decrease of only approximately 50% was observed from a summer high value of 22 μmol·g dry wt^{-1}. P_i showed a marked fivefold increase from a low value of 22 μmol·g dry wt^{-1} in summer to 106 μmol·g dry wt^{-1} in the winter. The total cellular concentration of NMR-visible P_i, Arg P, ATP, and ADP remained essentially constant, ranging from a summer high of 250 μmol·g dry wt^{-1} to a winter low of 191 μmol·g dry wt^{-1}. In the adductor muscle from winter adult scallops, the level of ATP was considerably higher (48 ± 8 μmol·g dry wt^{-1}) than in juveniles. The concentrations of Arg P and P_i were also higher than in the juveniles, and variable, ranging from approximately 90 to 280 μmol·g dry wt^{-1} for Arg P, with corresponding inverse values for P_i. It should be noted that the levels of these metabolites in adult muscle tissue did not change over the 2-h measurement period in the NMR tube. The mean of the ratio ATP β:(ATP γ + ADP) was not significantly different from unity (0.99 ± 0.05), indicating that ADP levels in the muscles of these adult scallops were not detectable by NMR.

Changes in Phosphorus Metabolites in Response to Short-term Anaerobic Stress

Juvenile scallops harvested in any season could be maintained in a stable physiological condition in the NMR tube for at least 10 h with no change in phosphorus metabolite levels. However, when the medium was deoxygenated, prompt changes were observed. The extent of hydrolysis of Arg P was strongly dependent upon the time of year that the scallops were collected. In the summer scallops (July–October), there was very little change in the levels of Arg P, P_i, or ATP during 5 h of hypoxia (Fig. 5). However, in the fall and winter scallops collected in November, December, and early April, the Arg P concentration was reduced, P_i increased, and ATP declined, the latter by an average of 7.4 ± 1.9% ($P = 0.001$) (Fig. 5 and Fig. 6).

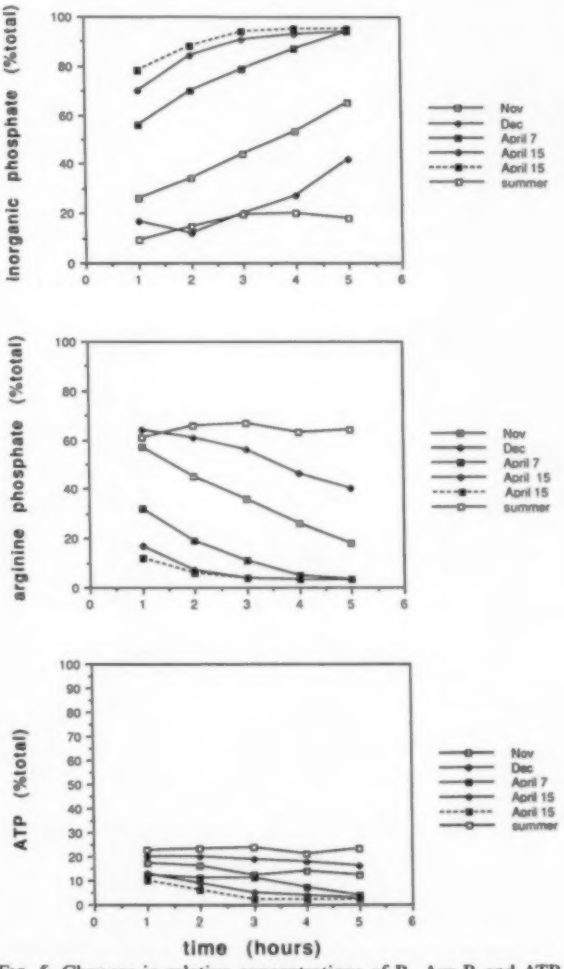

FIG. 5. Changes in relative concentrations of P_i, Arg P, and ATP (means, typical SD ± 6%) under exposure to hypoxia in juvenile scallops collected in summer (July–October), fall (November, December), and winter (April 7 and 15, before the spring bloom).

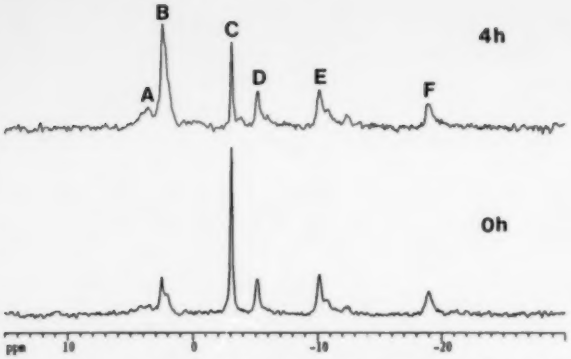

FIG. 6. Spectra recorded from juvenile scallops collected in December. Lower trace, 0 h: before hypoxic conditions applied; upper trace, 4 h: after exposure to 4 h of hypoxia. Resonance assignments: A, phosphomonoesters; B, P_i; C, Arg P; D, ATP γ/ADP β; E, ATP α/ADP α/NADH/NADPH; F, ATP β.

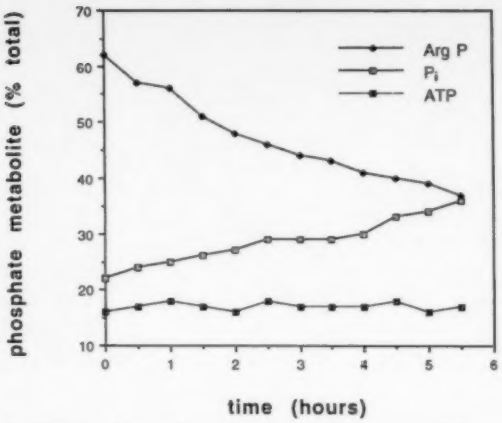

FIG. 7. Effects of hypoxia on relative concentrations of P_i, Arg P, and ATP in excised adductor muscle tissue from an adult scallop.

As expected, the summed concentration of P_i, Arg P, and ATP was unchanged during 5 h of anaerobic conditions in both season groups (two one-way ANOVAs, $P = 1.00$). Changes in the levels of phosphate metabolites under anaerobic conditions were also measured in the freshly excised adductor muscle of an adult scallop collected in June. The results (Fig. 7) demonstrated the same pattern of metabolic change as that which occurred in the fall and winter juvenile scallops. All intact scallops remained alive during the anaerobic exposure in the NMR tube. The summer and fall scallops that were returned to the aquarium remained alive for the week during which they were observed following the experiment. Winter scallops returned to the aquarium died within 24 h.

The intracellular pH values, as derived from the chemical shift of the peak for P_i, were 7.04 ± 0.19, 6.97 ± 0.12, and 6.69 ± 0.21 in juveniles collected in summer, fall, and winter, respectively. The only difference of statistical significance was between summer and winter scallops (independent t-test, $P = 0.04$). Under hypoxic conditions (over a 4- to 7-h period), there was no change ($P = 0.2$) in the intracellular pH of scallops collected in the summer months. Anaerobic conditions significantly (paired t-test, $P = 0.04$) reduced the intracellular pH of the fall and winter scallops to 6.38 from an initial value of 6.80. In the excised adult muscle (collected in June), the pH fell from 7.0 to 6.9 (5 h of hypoxia) to 6.8 (6 h of hypoxia).

In the laboratory study, intracellular pH was significantly different in the four treatment groups (mean 7.07 ± 0.13), similar to the intracellular pH of juveniles collected in summer and fall. Under hypoxic conditions the pH decreased ($P \leq 0.001$) to a mean value of 6.82 ± 0.07, the decrease being unaffected by feeding regime ($P = 0.5$) or temperature ($P = 0.2$).

Results from the laboratory study on the effects of temperature and nutrition on phosphorus metabolite levels showed that the temperature regime (15°C or 11–6°C) did not significantly influence the percentage composition, or concentrations per unit dry weight, respectively, of either P_i (MANOVA, $P = 0.5$ and 0.6) or Arg P ($P = 0.4$ and 0.9) in juvenile scallops, and hence the results from the two temperature treatments were pooled. The feeding experiment (Table 2) showed, when standardized to dry weight, that Arg P was higher in the scallops that had been fed than in unfed scallops ($P = 0.023$), from both temperature regimes. Levels of P_i and ATP, however, were the same in all treatment groups (respectively, $P = 0.3$, 0.3 for feeding; 0.4, 0.1 for temperature), resulting in a significant difference in the total of P_i, Arg P, and ATP between fed and unfed scallops.

TABLE 2. P_i, Arg P, and ATP in juvenile scallops maintained in the laboratory under different feeding regimes, expressed as percentages of the total of NMR-visible P_i, Arg P, ATP, and ADP (means ± 1 SD) and as μmol·g dry wt^{-1} (in parentheses).

Feeding regime	P_i	Arg P	ATP	n
Fed	11 ± 3	67 ± 2	22 ± 4	6
	(20 ± 7)	(121 ± 40)	(39 ± 14)	6
Unfed	18 ± 3	60 ± 6	22 ± 5	6
	(21 ± 6)	(69 ± 20)	(25 ± 10)	6

Under hypoxic conditions for 5 h, scallops from all treatments showed increased levels of P_i and decreases in Arg P (Fig. 8). In all cases, ATP decreased by a small but significant amount that was unaffected by all treatments (paired t-test for pooled results, $P = 0.015$). There was no significant change ($P = 0.6$) in the total NMR-visible phosphates. Neither the temperature nor the feeding regime had any effect on the magnitude of the changes in either P_i (MANOVA, $P = 0.9$ for temperature and $P = 0.05$ for feeding) or Arg P ($P = 1.0$ and 0.07). However, Arg P (micromoles per gram dry weight) decreased by a greater amount (61 ± 16%) in unfed than in fed scallops (26 ± 9%). There were no significant differences between the ratios of NMR-visible ATP:(ATP + ADP) under the experimental conditions of temperature ($P = 0.9$) and nutrition ($P = 0.8$), and this ratio was not significantly affected by 4 h of hypoxia (paired t-test, $P = 0.9$). The mean AEC calculated from the NMR spectra was 0.73 ± 0.10 for all treatment groups of juvenile scallops in the laboratory experiment, compared with 0.99 ± 0.05 for the excised adductor muscle of adult scallops.

Discussion

Phosphorus Metabolite Levels as Indicators of Overwintering Condition

The intracellular concentrations of ATP and related adenylates have been examined in a wide range of organisms including molluscs (Ivanovici and Weibe 1981), and changes in ratios of adenylates have been used as apparently reliable biochemical indicators of energy status in organisms exposed to nonoptimal conditions. For example, decreases in the AEC to values of 0.5–0.7 have been reported with prolonged starvation in the sea anemone *Aiptasia pulchella* (Steen 1986) and under anoxia in the sea anemone *Bunodosoma cavernata* (Ellington 1981), the estuarine bivalve *Trichomya hirsuta* (Ivanovici 1977), and the cockle *Cardium edule* (Gäde and Meinardus 1981). However, the cellular concentrations of adenylates are stabilized by biokinetic controls (Ellington 1981) so that even when energy reserves become severely depleted, any resulting decreases in ATP levels may not be associated with changes in the ratio of adenylates. This position is supported by our results showing that the decline in levels of both Arg P and ATP from summer through fall to winter that we observed in juvenile scallops collected in the field occurred without any change in the AEC. Considering the critical role of adenylates in all aspects of cellular metabolism, this is not surprising. In fact, acclimation to slow seasonal changes in temperature and food availability would be expected to facilitate maintenance of the "normal" or "preferred" adenylate ratio even in late winter. In this connection, we should note that the AEC in juvenile scallops must be under very tight control, since our results show that even the acutely nonoptimal conditions of oxygen deprivation for 5 h did not elicit a measurable change in the AEC of winter juveniles. Also, it should be noted that similar results were obtained in our laboratory study of juvenile scallops where neither starvation nor 5 h of hypoxia had any measurable effect on AEC. The mean value, 0.73, was intermediate in the range specified by Ivanovici and Weibe (1981), although there was a small decrease in the percentage of ATP under hypoxia (Fig. 8). Other results similar to ours, showing minimal changes in AEC, have been reported with the foot muscle of the bivalve *Cardium tuberculatum* (Gäde 1980), the pedal retractor muscle of the whelk *Nassarius coronatus* (Baldwin et al. 1981), and the ventricle of the whelk *Busycon contrarium* (Ellington 1983b).

Although there have been various reports of stressful situations producing changes in the AEC in molluscs (Ivanovici and Weibe 1981), the AEC is probably a less sensitive indicator of stress in molluses than the level of Arg P or the ratio Arg P:P_i because the levels of ATP are maintained at the expense of Arg P. Indeed, in our experiments, Arg P concentrations, particularly in relation to P_i, changed in response to treatments that caused no measurable change in AEC. The AEC value measured by NMR in juvenile scallops (0.73) is lower than that which we measured in the adductor muscle of adult scallops (0.99). The latter value is generally higher than the values measured by in vitro assay techniques in the muscles of other molluscs (e.g., Rainer et al. 1979). Higher values of AEC in the adductor as compared with other body tissues have been reported in other molluscs (Wijsman 1976), and it is likely that the inclusion of other body tissues results in lower estimation of AEC in the juveniles. As noted below, should some of the ADP in the adductor muscle be bound to actin (Graham et al. 1986), NMR measurements on excised muscles would underestimate the concentration of ADP, leading to overestimation of AEC, as NMR experiments of this type measure only compounds that are mobile. For example, the appearance of a peak in the phosphodiester region of the spectrum of adult muscle tissue extract, which was not apparent in that of whole muscle, was probably the result of the extraction of a phospholipid compound from a bound state, such as in a membrane (Lundberg et al. 1990). In our study, ADP levels in the excised tissue of *P. magellanicus* were below the limits of detection by NMR, but when the tissue was extracted the presence of an ADP peak may have been the result of an extraction-related hydrolysis artifact or of mobilization from actin. A similar result that may have been due to actin binding has been reported in the muscles of other scallops (Graham et al. 1986). However, this probably results

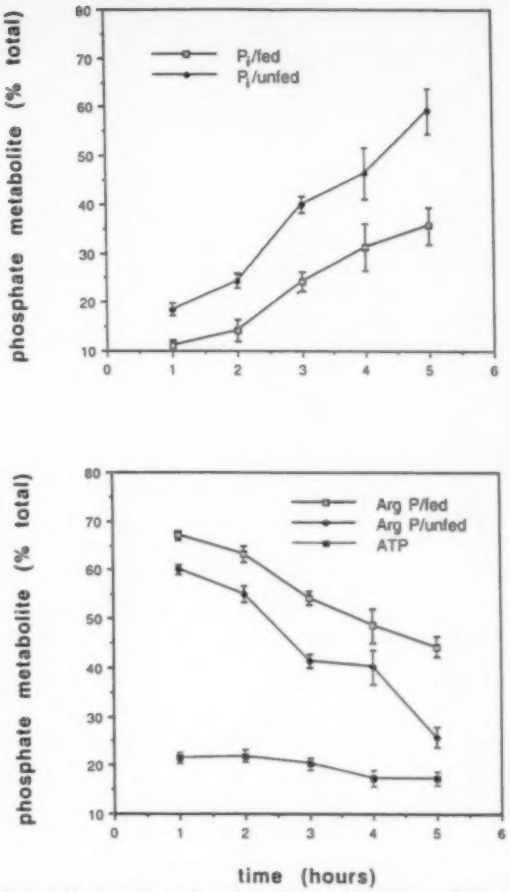

FIG. 8. Effects of hypoxia on relative concentrations of P_i, Arg P, and ATP (means ± SE) of juvenile scallops maintained in a fed or unfed condition for 6 wk. Both nutritional level and temperature of the seawater supply were under laboratory control in a 2 × 2 factorial experiment (see Materials and Methods). Results at different temperatures (15°C, 11–6°C) showed no significant differences and have been pooled (see text). The ATP values apply to both fed and unfed treatment groups. Note that total NMR-visible phosphates remained constant over the period of hypoxia in both fed and unfed treatment groups.

in only a minor error in quantitation according to previously published values (Livingston et al. 1981) showing a close correspondence between the concentration of high energy phosphates in muscle of adult *P. magellanicus* determined by NMR and values obtained using chemical assay.

Scallops have higher concentrations of Arg P and the adenylates than other, more sessile bivalves, probably as an adaptation to a more active existence. For example, the values obtained here for *P. magellanicus* are at least an order of magnitude higher than those of species of *Mytilus* (Zs.-Nagy and Ermini 1972) and various other marine molluscs (Nicchitta and Ellington 1983). It should be evident at this point that the implicit assumption of a causal and direct relationship between levels of high-energy phosphates and scallop condition is not in question. However, in the context of overwintering stress, it may be useful to distinguish between changes in phosphate metabolite levels that result from acute short-term stress such as bursts of muscular activity in response to predators, as compared with changes that are associated with more slowly incrementing stress factors such as a seasonal variation in temperature and the availability of nutrients.

The major role of nutrient supply in maintaining energetic status is supported by our laboratory study of temperature and nutrition effects which showed that food availability influenced the amount of Arg P in juvenile scallops kept under laboratory conditions. Nevertheless, the experiment did not clarify the seasonal differences in the extent of net Arg P hydrolysis under hypoxic conditions that were observed in scallops harvested directly from the field. The rate of Arg P hydrolysis in laboratory-held scallops was not affected by feeding or by temperature. The scallops for this study, collected in October, did not use Arg P when subjected to 4 h of hypoxic exposure 1 d after collection, but after a 6-wk sojourn in the aquarium system, net hydrolysis of Arg P during hypoxia was observed in all scallops. Evidently, attempts to simulate summer field conditions by heating the seawater to 15°C, and feeding, were unsuccessful. Fed scallops appeared to be ingesting the food particles, as judged from the production of feces, but it is possible that the quality of this food source was not comparable with that of natural phytoplankton populations. Note that, although the concentration of Arg P was lower in unfed scallops, this was not associated with a higher concentration of the breakdown product, P_i, suggesting that the latter had been excreted. Loss of P_i under starvation conditions has been reported in other animals (Steen 1986).

Seasonal Influences on Aerobic–Anaerobic Metabolism

There are two primary means by which ATP is regenerated in molluscs: dephosphorylation of Arg P and the breakdown of glycogen (Gäde 1983; Hummel et al. 1989). According to Hochachka et al. (1983), there are probably many species of molluscs that use the phosphagen system almost solely to power muscular work, and anaerobic glycolysis mainly for recovery, although this is certainly not the case for all molluscs (Wiseman et al. 1989). However, Livingston et al. (1981) reported that 70% of the anaerobic energy production during burst contractile activity in the phasic adductor muscle of adult *P. magellanicus* was derived from the breakdown of Arg P. It has been observed that swimming activity is greatly reduced in juvenile *P. magellanicus* during winter (Manuel and Dadswell 1991; B.G. Hatcher et al., unpublished data), but this could be the result of depressed metabolic activity at low temperature, rather than a consequence of the relatively low levels of Arg P available for regeneration of ATP in these scallops.

We found that juvenile *P. magellanicus*, collected in the summer months, showed no change of Arg P levels under anaerobic conditions, so presumably ATP was being regenerated via glycolysis. The marked hydrolysis of Arg P under anaerobic conditions in juvenile scallops collected in winter could be a reflection of low glycogen reserves in scallops during winter, and hence a reliance on Arg P as an energy source, rather than glycolysis as suggested by Hummel et al. (1989). In adult *P. magellanicus*, muscle glycogen normally peaks after the spring bloom and is then transferred to the gonad prior to spawning in fall (Robinson et al. 1981). Hence the low muscle glycogen recorded during winter reflects postspawning exhaustion of reserves and low nutrient availability (Gould et al. 1988). In juveniles, since reproductive effort is not a factor, the energetic status may be considered to be primarily dependent upon environmental conditions, particularly the availability of nutrients.

Previous ^{31}P NMR studies in vivo of molluscs have shown decreases in Arg P levels and intracellular pH, increases in P_i, and lesser changes in ATP levels, in response to osmotic stress in abalone (Higashi et al. 1989) and hypoxia in abalone (Tjeerdema et al. 1991) and mussels (Fan et al. 1991). In our experiment, the enhanced net utilization of Arg P under anaerobic conditions, which was observed in fall and winter scallops as compared with summer scallops, occurred in conjunction with reduced levels of high energy phosphates, and increased levels of P_i. In addition, the chemical shift of the P_i signal is sensitive to changes in intracellular pH (Fig. 2). Intracellular pH in *P. magellanicus*, measured by NMR in vivo in the summer and fall juveniles, and in adult muscle tissue, was found to be 7.0. This is low compared with typical values (approximately 7.5) reported for intact abalone (Tjeerdema et al. 1991) and mussels (Fan et al. 1991), although it is very similar to the pH measured in excised muscles of other molluscs (Ellington 1983a, 1983b; Chih and Ellington 1985). Under hypoxic conditions for 5 h, juvenile scallops collected in summer months maintained constant pH as well as constant Arg P levels. By contrast, in fall and winter scallops, hypoxia caused marked reduction in both intracellular pH and Arg P levels. Similar changes were reported (Fan et al. 1991; Tjeerdema et al. 1991) for intact abalone and mussels and were attributed to H^+ production from anaerobic glycolysis. Buffering by H^+-consuming fermentation reactions was also postulated as a mechanism preventing further decline of pH, and it is possible that such a mechanism is enough to maintain constant pH in summer scallops. Buffering systems and active H^+ pumps both serve to regulate intracellular pH in other animal cells (Roos and Boron 1981). Fluctuations in intracellular pH during anaerobic metabolism have also been found in excised molluscan muscle. Chih and Ellington (1985) attributed a transient initial increase in pH during rapid contractile activity in the adductor muscle of the bay scallop *Argopecten irradians* to H^+ consumption during Arg P hydrolysis and the subsequent pH decline to H^+ production from glycolysis. A pH decrease under conditions of anoxia over similar time periods (Ellington 1983a, 1983b) has been attributed to accumulation of acidic end products, such as succinate, from glycogen breakdown (de Zwaan and Wijsman 1976; Chih

and Ellington 1985). It is possible that this decrease in pH served to inhibit phosphofructokinase and hence glycolysis (Ellington 1983b), resulting in an increase in Arg P hydrolysis such as we observed in the fall and winter scallops.

Although marked changes in the adenylates have been noted during anaerobic metabolism in other molluscs, including scallops (Grieshaber 1978), there have been other cases where no appreciable changes in the adenylates have been observed in bivalve muscle tissue (e.g., Gäde 1980). Studies of abalone (Tjeerdema et al. 1991) and mussels (Fan et al. 1991) attributed the observed effects of hypoxic conditions (decreased Arg P levels, decreased pH, increased P_i, lesser changes of ATP) to blockage of mitochondrial oxidative phosphorylation and ATP regeneration by anaerobic glycolysis producing excess H^+. The differences observed between season groups in our study make it apparent that the season of collection is an important consideration in accounting for some of the previously observed variability. The proportion of Arg P hydrolyzed by fall and winter juveniles, and by the muscle tissue of adult scallops during environmental anaerobiosis in our experiments, was similar to that reported in the previous in vivo NMR studies mentioned above and considerably more than that reported for the tissues of other molluscs by Gäde (1983) (e.g., 23 and 21% in the adductor muscles of *Mytilus edulis* and *Lima hians*, respectively, and 10% in *Cardium tuberculatum*). However, Gäde (1983) did report a higher utilization rate in active species over their sessile counterparts. From this discussion, it is evident that an examination of intracellular levels of phosphorus metabolites, and changes in levels, can only yield limited information on the critically important quantitation of rates of formation and hydrolysis of Arg P and the adenylates in bioenergetic processes. Further work in this area using NMR methods such as saturation transfer might clarify at least some of the kinetic aspects of phosphorus metabolism in scallops.

NMR spectroscopy is currently used extensively in medically related physiological and biochemical studies. It provides an ideal means for in vivo monitoring of intracellular processes, particularly the critical energy-related processes involving phosphate transfer reactions. We expect that this very powerful experimental tool will be used increasingly in a wide variety of fundamental and applied areas of marine biology. From the standpoint of our use of ^{31}P NMR as applied to shellfish aquaculture research and development, we conclude that the levels of Arg P and P_i in juvenile scallops, as measured by ^{31}P NMR in vivo, are sensitive indicators of seasonal variation in biochemical energy reserves, particularly when the organisms are subjected in the laboratory to the further stress of oxygen deprivation. In scallops and other organisms that use Arg P to replenish ATP, such measurements of changes in Arg P and P_i levels in response to laboratory-induced hypoxia might prove to be generally useful indicators of quality in marine environments being tested as potentially suitable sites for "grow out" and/or maintenance of brood stocks.

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PERSPECTIVES

Cooperative Fisheries Management Involving First Nations in British Columbia: An Adaptive Approach to Strategy Design

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In 1991, a substantial program of cooperative salmon fishery management involving First Nations was initiated in British Columbia. This paper considers how ongoing cooperative management initiatives in this fishery could be designed and implemented. The process discussed in the paper could be viewed as an adaptive management experiment in institutional design for cooperative management. First, sets of fundamental objectives for cooperative management and strategies for achieving these objectives are developed. The methods for structuring objectives and developing strategies are drawn from decision analysis practice. Then the actual experience in implementing a version of one of the strategies during the 1992 Fraser River sockeye salmon First Nations fishery is discussed. The highly publicized and controversial events of the 1992 season are instructive about how cooperative management should be implemented in other contexts.

En 1991, un important programme de gestion coopérative de la pêche au saumon a été mis sur pied en Colombie-Britannique, avec la participation des Premières Nations. Le présent article traite des possibilités d'élaboration et de mise en oeuvre de programmes de gestion coopérative permanents pour cette pêche. Le processus décrit ici pourrait être considéré comme une expérience adaptative dans l'élaboration institutionnelle de programmes de gestion coopérative. Tout d'abord, on formule une série d'objectifs fondamentaux propres à la gestion coopérative, de même que des stratégies pour atteindre ces objectifs. Les méthodes de détermination des objectifs et d'élaboration des stratégies sont établies d'après l'analyse des décisions. On traite ensuite de l'expérience pratique acquise lors de l'implantation de l'une de ces stratégies, en 1992, dans la pêche autochtone de saumon rouge du fleuve Fraser. Les événements très médiatisés et controversés de 1992, sont riches en enseignement sur la façon dont la gestion coopérative devrait être mise en oeuvre dans d'autres contextes.

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In 1991, the Canadian Department of Fisheries and Oceans (DFO) initiated a substantial program of cooperative salmon fishery management projects with native Indian bands (referred to here as First Nations) in British Columbia. Cooperative fisheries resource management or "comanagement" is defined as negotiated agreements and other arrangements made between fishermen and government agencies responsible for fisheries management (McCay 1992). The 1991 projects, which involved over $11 million in funding and some 150 contracts with First Nations, represent one level in a trilevel approach linking fisheries management to land claims negotiations (Pearse 1992). Even though Canada has been negotiating land claims with First Nations in British Columbia since at least 1973, progress toward overall land claims agreements has been slow (Turpel 1991). Nevertheless, there is general recognition that land claims agreements will include a significantly increased involvement by First Nations people in the stewardship of fishery resources. For practical as well as ethical reasons, DFO has begun to develop arrangements for cooperative management of fisheries with First Nations in advance of the final land claims agreements (DFO 1991).

While the desirability of greater involvement by First Nations in fisheries management is recognized, exactly what form this involvement should take and how it should be accomplished remain wide open questions. Many forms of comanagement are possible, differing mainly in the degree of shared responsibility. These range from contracting with First Nations people to undertake routine monitoring and technical activities at one extreme, to complete sharing of decision-making responsibility for key issues at the other extreme (Pinkerton 1989; McCay 1992). DFO has little experience that would be of direct help in designing strategies for involving First Nations in fisheries management. Such profound changes in organizational and resource management practice can pose serious difficulties, both for science-based organizations

such as DFO and for other interested parties (Downs 1967). The lack of accepted operating procedures to cope with the changing environment creates a need for innovative approaches (Behn 1988).

When faced with complex resource management decisions in uncertain environments, resource ecologists often advocate "adaptive management," which involves structured management experiments to learn about ecological systems (Holling 1978). Adaptive management is also relevant for learning about the implications of new institutional approaches for resource management (Walters 1986). Creating new comanagement regimes where there is no history of such power-sharing is a context where adaptive approaches that provide opportunities for learning over time would be extremely useful. More broadly, fisheries comanagement could also be viewed as a new approach to managing shared public resources that will require careful development of strategies to achieve successful implementation.

Because of the emerging importance of comanagement, Westwater Research Centre decided to research possible designs for fisheries comanagement involving DFO and First Nations in British Columbia. The results of our research form the basis for two papers. The present paper considers the design of alternative strategies for comanagement and the conflicts that arose in implementing comanagement during 1992. A companion paper considers how the experience in fisheries comanagement elsewhere can be informative in shaping new strategies in British Columbia (R.K. Paisley, M. Healey, and T. McDaniels, in preparation).

The events described in this paper can be viewed as an experiment in adaptive management for design of fisheries comanagement. The paper begins by carefully developing the fundamental objectives that should guide salmon comanagement in British Columbia. Then the organizational context in which comanagement would be implemented is discussed. Next, four strategies comprising four generic approaches to comanagement are discussed. The four strategies differ in several key control variables, including the degree to which real decision-making powers are delegated to First Nations and the degree to which programs are designed to help DFO and First Nations learn about cooperative management. Implementation of a version of one of the four strategies during the 1992 salmon fishing season provides the experimental result that allows managers to learn from the initial strategy development and implementation process. The lessons of the highly publicized, controversial events of 1992 are instructive for future comanagement initiatives in British Columbia and for comanagement effects elsewhere.

Objectives for Cooperative Management

A key first step in developing strategies for comanagement, or any other complex decision, is to clarify the fundamental objectives the strategies are intended to achieve. Clarifying objectives can be particularly useful in public resources management decisions that involve many stakeholders and conflicting views about priorities (Keeney 1992). Yet, in most organizational contexts, direct focus on objectives as a basis for decisions is rare. Instead, decisions involving modest changes in the status quo are typically made on the basis of standard operating procedures (March and Simon 1958) and proceed in incremental steps (Lindblom 1959).

TABLE 1. Initial objectives of DFO's cooperative management program implied by program documentation.

Overall Objective: increase benefits from British Columbia fisheries to First Nation people and all Canadians through cooperative management ventures
Improve economic benefits from fisheries
Improve profit and stability in industry
Improve economic self-sufficiency of First Nations
Improve social benefits from fisheries
Increase self-sufficiency and self-esteem among First Nations
Develop trust and respect
Educate the public
Make progress in land claim negotiations
Improve fisheries health
Rehabilitate, conserve, and enhance productive fish habitat
Manage fisheries well

Although standard procedures and incrementalism may work well in organizations whose goals are not changing, they are of less help when major shifts in organizational practice or philosophy are contemplated.

The approach adopted here for structuring objectives is termed multiattribute value assessment, as applied in public policy contexts (Edwards and von Winterfeldt 1987). The approach entails detailed interviews to represent the multiple objectives important to various groups in a policy decision. The sets of objectives from each group are integrated into an overall set of objectives that reflects all viewpoints. The approach can be either qualitative or quantitative. If qualitative, the objectives serve as the basis for developing alternatives that are broadly attractive from the viewpoints of those interviewed. The objectives are also useful for improving communication, diagnosing conflict, and identifying important information (Keeney 1992). If quantitative, the analysis involves establishing measures for objectives and then eliciting priorities among objectives in the form of mathematical utility, or value, functions (Keeney 1988). A qualitative approach is adopted here. The methods discussed in Keeney (1988) regarding objectives for problems of public interest and the elicitation approach illustrated in Keeney and McDaniels (1992) for developing objectives for a particular organization were both adapted for use here. The process of structuring the fundamental ends (objectives) of interest and then developing strategies that could potentially achieve those ends has been referred to as "value-focused thinking" (Keeney 1992).

Initial Objectives Implied by Program Documentation

As a preliminary step in structuring objectives for cooperative management, we reviewed the documentation for DFO's 1991 program. The documents provide some insight into objectives for overall cooperative management activities, even though objectives of the program are not specifically discussed. We derived a list of implied objectives for the program (Table 1) from three internal documents and one press release provided by DFO (DFO 1991).

Judging from this written documentation, the overall objective for the program from DFO's viewpoint is to increase benefits from British Columbia fisheries to First Nations and all Canadians through cooperative management ventures.

More specific objectives drawn from the documentation fall into three general categories: economic benefits, social benefits, and fisheries health.

The documentation that provided the list of objectives in Table 1 suggested that DFO sees cooperative management as primarily involving First Nations people taking on tasks that are a normal part of DFO's fisheries management programs. Although there is some attention paid to First Nations' views and traditional approaches, the dominant impression conveyed by the documents is that DFO expected to set the agenda for fisheries management and then to negotiate cooperation with First Nations. We felt it was important to explore the objectives First Nations also had for cooperative management and to discuss openly with DFO staff the fundamental objectives of the program. Consequently, we proceeded, through a series of focused interviews with knowledgeable stakeholders, to structure explicit objectives that could serve as a basis for new cooperative management activities.

TABLE 2. Preliminary objectives hierarchy for cooperative management specialist.

Overall objective: design the best possible cooperative management programs
Improve equity
For Native interests
For future generations
Improve local involvement
Use local knowledge
Use local protection
Use local decisions
Improve stock health
Increase diversity of stocks
Increase productivity of stocks
Improve economic well-being
Fisheries harvest and production employment
Management employment

Interview Process

The interview process for structuring objectives began by identifying individuals who could speak about the views of particular stakeholder groups, who were knowledgeable about cooperative management, and who would be willing to speak freely and meaningfully. A number of groups were contacted (in government and among First Nations) and asked to suggest people. Four individuals were selected for interviews. These included two consultants knowledgeable about fisheries cooperative management, one of whom is a fisheries specialist and the other a cooperative management specialist. The other two people were a representative of DFO who is knowledgeable about the design and administration of cooperative management initiatives from that organization's viewpoint and a Chief of the Chilliwack Band who is also a lawyer, a fisherman, and deeply involved in fisheries issues on behalf of First Nations political organizations.

The key stakeholders considered here are clearly DFO and First Nations in British Columbia. Other important stakeholder groups also have a major interest in cooperative fisheries management questions; these include commercial and sport fishermen, the British Columbia government, and average citizens who are the resource owners. One could also differentiate between representatives of DFO from the Pacific Region and from the Central DFO administration in Ottawa, as well as differentiate between coastal and interior First Nations. All these groups have different interests that could shape underlying objectives for comanagement, as well as influence its success.

Even though there are several possible perspectives, the emphasis here on the viewpoints of DFO, First Nations, and related groups (the consultants) is justified because these were the groups actually involved in developing and advising on comanagement programs. In the interviews, participants identified the fundamental objectives that should be relevant from all perspectives, not just their own. Previous experience suggests that interviewing several additional parties would have been unlikely to have identified new fundamental objectives not mentioned by these participants. Most or all groups would likely agree on the basic set of underlying objectives for comanagement, even though they may differ substantially on the relative priorities among objectives (Keeney 1992). Other groups might also have concerns about related issues, such as fisheries allocation. As discussed later, fish allocation could be considered a separate, longer term issue. Some might also question the number of individuals interviewed. Developing objectives is not a matter of sampling large numbers of individuals. Instead, it involves in-depth interviews with fewer people to get detailed, thoughtful information. Four detailed interviews is a reasonable number for a first attempt at objectives (Keeney and McDaniels 1992). In sum, this initial attempt at structuring objectives focused on the broad concerns of DFO and First Nations people. The importance of differences in perspectives within DFO and in perspectives among coastal and interior First Nations is discussed later.

The four people chosen for interviews were interviewed individually (except in the interview with the DFO representative, in which two DFO staff were present and both participated). Before the interviews, we developed a protocol involving a series of steps that were followed in each interview. The first step was to explain what the interview was intended to achieve. Those interviewed were then asked to discuss the factors that should be important in guiding the design of cooperative fisheries management initiatives involving First Nations in British Columbia. Two devices were used to encourage creative thinking. Each person was asked to provide a scenario of what good cooperative management would look like 20 yr hence. They were also asked to provide a disastrous scenario for fisheries cooperative management. These approaches were useful in getting the individuals to think beyond the status quo and helped reveal implicit objectives.

The interviews were conducted as in-depth, two-way discussions. Two study team members interviewed the subjects, and both took notes and probed for underlying objectives and motivations. We strove to derive a set of structured objectives that reflected all the factors viewed as broadly important for designing comanagement. In particular, we worked to distinguish ends from means, to discuss the relationships between objectives, and to clarify definitions of what specific objectives meant. In some cases, objectives were suggested and those interviewed were asked to comment on their relevance for cooperative management. No attempt was made to set priorities among objectives.

TABLE 3. Characteristics of success and failure in cooperative management 20 yr in the future, provided by cooperative management specialist.

Success	Failure
Currently weak stocks rebound	Many court cases
Diverse, productive year-round fisheries	Reduced productivity and diversity of stocks
Continual Native employment opportunities	Local anger over stock losses
High school students taking pride in local fisheries productivity	No local control, empowerment, or enforcement
Local monitoring and enforcement by Native/non-Native residents	Increases in closures due to pulp mill contamination
Local control and Empowerment regarding fisheries decisions	Legitimacy of local activities undermined
Traditional knowledge and decision approaches become recognized	Violation of local interest and knowledge by outsiders (floating lodges)
Agreement among all users for cooperation, not competition	People lose heart
No major conflict	

Individual Objectives

Three products were derived from each interview. These included a value tree, or hierarchy of objectives mentioned by the interviewee as important for cooperative management, and summaries of scenarios for cooperative management success and failure. The latter summaries were important in developing the set of objectives for each individual. To illustrate the insights about objectives derived from the interviews, we will review in detail the results of the interview with the cooperative management specialist. Subsequently, we will discuss the integrated results from all four interviews.

The cooperative management specialist works as a consultant on a variety of First Nations cooperative management initiatives. The interview involved a wide-ranging, in-depth discussion that lasted over 2 h. Table 2 summarizes the hierarchy of fundamental objectives that this person viewed as important in shaping cooperative management strategies. The specialist concurred that the overall objective was to design the best possible cooperative management programs. The objectives listed below the overall objective clarify what is important in designing the best possible programs. These fundamental objectives include increasing equity (for Native groups and for future generations), increasing local responsibility, increasing health of stocks, and improving community economic well-being. Each of these objectives has subdivisions that clarify what that objective means. Local involvement was viewed as a fundamental objective, but also one that provides a means for addressing the other objectives more readily. In this specialist's view, successful cooperative management would be impossible without local responsibility and extensive reliance on local information and knowledge.

Table 3 summarizes the cooperative management specialist's views on scenarios of success and disaster for cooperative management. The cooperative management specialist was asked to provide a summary of the content of newspaper articles under hypothetical headlines announcing cooperative management success (and failure) 20 yr hence. By comparing these two summaries, we can discover clues to objectives that had not been stated earlier and clarify attributes that are important in designing strategies. For example, the importance of increasing the productivity and diversity of stocks had not been clearly articulated as an objective until mentioned in the summaries. When asked, the cooperative management specialist concurred that these were important objectives.

A separate summary of objectives, or objective hierarchy, was elicited from each person interviewed. There were many similarities among the objectives identified. For example, they all discussed cooperative management as an important way to build understanding about First Nations' rights and responsibilities regarding fisheries resources. These rights will eventually be determined in concrete terms through treaty making and land claims negotiation, or through the courts. Nevertheless, cooperative management was mentioned as an important step in building mutual understanding, trust, and cooperative processes that would be needed to implement these eventual rights. Cooperative management was also seen by all participants as having the potential to contribute to greater protection of fisheries habitat and stock health. Both the fisheries specialist and the cooperative management specialist could articulate clear links between increased local responsibility as a result of cooperative management and the potential for improving the health of stocks. This link could occur through better use of local fisheries knowledge, use of local people for wider and more direct enforcement, and use of local approaches to resolve local conflicts over resource use. Finally, cooperative management was discussed as a way to build trust that could eventually lead to less rancor among stakeholders.

As well as these commonalities, there were some distinct differences. For example, representatives of DFO tended to see cooperative management as a way to address fundamental questions of fisheries management such as conserving and protecting stocks, creating economic benefits, and undertaking management strategies that achieve wide support. Yet, the links through which cooperative management could help achieve these objectives were not clearly articulated.

The First Nations individual saw cooperative management primarily as a means to achieve clean water and healthy fish.

In his view, cooperative management was a way to foster agreement among groups that have traditionally been adversaries, i.e., DFO, First Nations, and commercial and sport fishermen. He envisioned that their common interest was in protecting and enhancing the resource and that cooperative management was a means of identifying and building cohesion around that objective. His attention was focused on the nature of the cooperative management process as a means to achieve the fundamental objective of improved stock health.

A particularly emotive issue was the degree to which cooperative management was seen as a means to address directly the issue of First Nations title to fisheries resources. As mentioned earlier, the precise nature of First Nations rights over fisheries resources will eventually be defined in treaty-making activities and perhaps through the courts. In the interviews, the cooperative management specialist and the fisheries specialist, both of whom work extensively for First Nations, saw cooperative management as a means to educate people about the responsibilities of fisheries management and to start the process toward granting First Nations some degree of responsibility over the resource. Conversely, both the DFO administrator and the First Nations fisherman wanted to separate, at least conceptually, cooperative management from eventual resolution of land claims. In their eyes, it was important to avoid the implication that cooperative management led to specific definition and implementation of rights. They perceived that by encouraging learning and building trust, cooperative management would lead to more informed negotiation about First Nations rights, but would not establish those rights. None of the interviewees considered specific definition of First Nations rights to be a direct objective for cooperative management. We return to the issue of First Nations fisheries allocations later in this paper.

Combined Objectives

Table 4 provides a combined hierarchy of objectives for cooperative fisheries management. It includes all the objectives mentioned by at least one of the four people interviewed. In a number of cases, the objectives have been consolidated or slightly restructured to reduce redundancies. The intent is to provide a set of objectives that all would agree contains the factors important in designing cooperative management strategies. As mentioned earlier, the interviews did not consider the question of priorities among these objectives, so the order in which the objectives appear does not reflect their relative importance. Two of the objectives in the table (improving stock health and improving community economic well-being) are relevant for all kinds of fisheries management activities. Thus, it is not surprising that they are listed here as fundamental objectives for cooperative fisheries management. Two other objectives (improving equity and improving trust and cooperation) are concerned with how the benefits and responsibilities of cooperative management are shared. The remaining two objectives (improving community involvement and improving opportunities for learning) are concerned with how fisheries comanagement is conducted. The former is a basic tenet of how comanagement should proceed (Pinkerton 1988), while the latter is a basic tenet of adaptive management (Holling 1978).

Because the objectives in Table 4 summarize what participants believe is important to achieve with comanagement, the objectives should play a significant role when designing potential comanagement strategies (Keeney 1992).

TABLE 4. Preliminary combined hierarchy of fundamental objectives for fisheries cooperative management.

Overall objective: design the best possible cooperative management programs
Improving stock health
Improving stock diversity
Improving stock abundance
Improving community economic well-being
From fisheries harvest and processing
From fisheries management
Improving equity
For Native interests
Among Native groups
For future generations
Improving trust and cooperation
Among all users
Between First Nations and DFO
Improving community involvement
Using local knowledge
Using local protection
Using local decisions
Improving opportunities for learning
About fisheries
About science
About First Nations rights

Another important factor when designing strategies is the organizational context in which the strategies must be implemented, which is the subject of the next section.

DFO Organizational Concerns

It is important to recognize that the comanagement initiatives discussed here could involve some transfer of resources and authority from a regulator (DFO) to a regulatee (First Nations people). One might anticipate the possibility of resistance to such a change: it could possibly reduce the regulator's ability to carry out its conventional mandate, possibly reduce the power of existing staff, and possibly reduce budgets. Common sense (and organization theory) suggests that comanagement initiatives could face resistance from within DFO itself (Downs 1967). Consider the U.S. experience. Cooperative management in Pacific Northwest salmon fisheries evolved slowly after a decision by U.S. Federal Court Judge Boldt mandated that a specified share of the catch go to First Nations people (*US* v. *Washington*, 1974). Initially there was resistance by the State of Washington and other agencies to a cooperative approach. Cooperative management proved to be the only way to determine how the fish resources could be shared in practical terms (Cohen 1986).

Why, then, has comanagement proceeded as far as it has in British Columbia without DFO and other stakeholders being forced to implement it by the courts? Answers to this question should involve some understanding of organizational politics within DFO and events shaping the external environment within which DFO operates. This discussion can serve as a basis for identifying organizational concerns important to DFO in designing cooperative management regimes.

DFO's fundamental mandate, as set out in the *Fisheries Act*, has two components: conserve and protect fisheries resources and provide broad social and economic benefits from fisheries utilization (Canada 1985). In addition, DFO has both an established policy and a legal requirement (*R v. Sparrow* 1990) to provide fish for First Nations subsistence purposes, in priority after conservation and before all other uses. These three fundamental ends might be viewed as important organizational objectives for all fisheries management activities. These ends also fit well with the initial objectives implied by the program documentation for DFO's cooperative management programs (Table 1).

One might argue that these objectives have been accomplished passably well over the past 20 yr without comanagement initiatives. Where, then, is the impetus for a major change in organizational practice? To address this question, we conducted several interviews with individuals knowledgeable about organizational issues within DFO. These included the individuals interviewed regarding objectives as well as other key informants. Those interviewed concurred that the impetus comes from two forces, both originating with DFO headquarters staff in Ottawa. Of course, that is where the impetus for change of this nature must arise because DFO cannot delegate its authority, except as prescribed in legislation and as directed by its Minister. One force is the widespread recognition that management practice within DFO is being reformed. A number of initiatives are underway that are expected to change the practice of fisheries management in the Pacific Region. These will likely lead to a greater emphasis on local fisheries management, a concept in keeping with comanagement generally (DFO 1993). The second force is the growing political power of First Nations people in Canada. First Nations issues have received growing attention, exemplified by the long and well-publicized confrontation in Oka, Quebec, in 1990 (Taylor et al. 1990) as well as the importance of First Nations in debates over changes in the Canadian constitution (Janigan 1992). Another contributing force could be the recognition that First Nations have obtained substantial power over fisheries resources in the Pacific Northwest states, following the Boldt decision.

The Minister and senior officials of DFO in Ottawa have made public commitments to British Columbia First Nations that cooperative management will proceed with DFO headquarters' full support (DFO 1991). The obvious reasons for these commitments would be to prepare for the inevitability of land claims settlements involving fisheries, to respond to the growing political power of First Nations, to encourage the process of reform within DFO, to avoid possible litigation, and to build a cooperative rather than a confrontational environment among resource users.

According to those interviewed, the pathway followed to create change in DFO's organizational practice has been in keeping with that recognized in much of the writing on organizational cultures. An external change has occurred that has dramatically altered the organization's environment; organizational leaders, or "champions", have mandated the need for change; examples of how the changes could occur are created, and the organization's practices respond (Peters 1987). Yet, for the organizational changes to proceed effectively, the potential threats they pose to existing organizational practice must be recognized and appropriately managed. The impacts of comanagement initiatives on DFO in the Pacific Region must be explicitly recognized and mitigated if necessary. Strategy alternatives should, therefore, be developed that involve trade-offs regarding these potential organizational impacts and achievement of cooperative management's fundamental objectives.

Strategies

Strategies for cooperative fisheries management are approaches by which the objectives outlined above can be achieved and organizational concerns can be managed. Because DFO controls budgets and has responsibilities for fisheries management, it is important to focus on activities that DFO could consider as components of cooperative management strategies and how First Nations are likely to respond to such initiatives. As currently practiced, cooperative management in British Columbia is largely a series of individual projects, funded by DFO, in which First Nations undertake specific fisheries management activities. This seems to be a versatile administrative structure for fisheries cooperative management and one we will use to view the specific activities that could be undertaken. The crucial issues shaping the overall success of fisheries cooperative management are how such projects are selected, designed, and administered.

Policy Levers Available to DFO

A first step in developing strategies was to clarify the "policy levers" under DFO's control that would affect cooperative fisheries management, and how these might influence the involvement of First Nations people. During the interview process, in-depth discussions were held with participants to clarify the factors affecting First Nations involvement in fisheries cooperative management. Working with the fisheries consultant, we developed an influence diagram of the factors affecting First Nations involvement (Howard 1989). In such a diagram, an arrow running from one variable to a second simply means that the outcome of the first variable has influence on the outcome of the second. The variable selected to represent First Nations involvement was "the number of First Nations people meaningfully involved in fisheries harvests and management." We asked the specialist what factors would influence that variable, and in turn the factors that would influence those variables. The resulting influence diagram (Fig. 1) shows that the number of First Nations people directly involved in fisheries harvest and management would be influenced by (1) the economic benefits from fisheries harvest and management, (2) the degree of local responsibility for determining fisheries projects and patterns, (3) the degree that participants feel local control, and (4) the degree to which there is reliance on traditional knowledge and local habitat protection. These are in turn influenced by a number of variables, most important of which are the three variables identified in the figure as being under DFO control. These include DFO fish allocation processes, the degree of delegated responsibility, and the amount of money spent by DFO on projects run by First Nations. By allocating fish to First Nations, DFO could increase the incentives to participate in the fishery as well as the incentives to undertake long-term management and enhancement. By delegating responsibility, DFO could increase First Nations' sense of control, as well as building trust. By allocating financial resources, DFO could make it possible for First Nations to carry out their responsibilities.

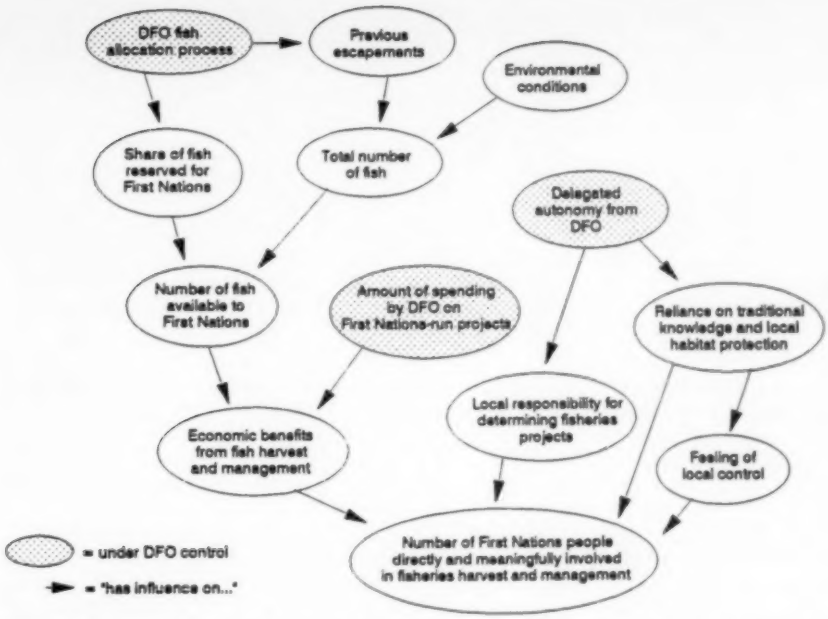
DFO fish allocation process
Previous escapements
Environmental conditions
Share of fish reserved for First Nations
Total number of fish
Delegated autonomy from DFO
Number of fish available to First Nations
Amount of spending by DFO on First Nations-run projects
Reliance on traditional knowledge and local habitat protection
Local responsibility for determining fisheries projects
Economic benefits from fish harvest and management
Feeling of local control
Number of First Nations people directly and meaningfully involved in fisheries harvest and management
= under DFO control
= "has influence on..."

FIG. 1. Influence diagram of factors affecting local First Nations involvement in fisheries harvest and management.

These three variables are, in essence, the policy levers available to DFO to shape fisheries comanagement strategies.

Range of Options

DFO's latitude in considering a range of actions in these areas is constrained by existing administrative arrangements, lines of authority, and availability of budgets. Consider first the issue of allocation of fish, which in this context refers to the First Nations share of total fish production. In our interviews to structure objectives, both the DFO representatives and the First Nations person emphasized that allocation of fish should not be a component of cooperative management activities, particularly in initial years. The reason is straightforward. Allocation of fish is a highly charged political and economic issue. Including it as an aspect of cooperative management strategies at this early stage would evoke strong opposition from other fish user groups, who would perceive that First Nations fishing interests were gaining ground at their expense. Both the DFO representatives and the First Nations spokesperson stressed the need to develop cooperative processes as a means for building trust and fostering learning as the principle concerns for cooperative management in the near term. Yet, all participants recognized that allocation of fish will eventually be necessary to provide real incentives, responsibility, and substance in moving beyond the first steps in comanagement.

Budgets available for cooperative fisheries management are also an area in which DFO does not have great latitude. In the first year of activities, over $10 million was allocated to cooperative fisheries management projects and senior decision-makers seem prepared to allocate a considerable share of the regional budget to cooperative management projects. Yet, given an annual budget allocation, DFO program administrators would seek projects up to the spending limit.

How the available funds are allocated and how the projects are carried out are the areas in which DFO has the greatest range of opportunities for creating attractive and workable strategies. Figure 1 shows that delegating responsibility to First Nations could have at least two important dimensions: determining the fisheries management projects that go forward and determining how projects are conducted. Yet, increasing the degree of First Nations autonomy in both selecting and carrying out projects poses conflicts with DFO's traditional view of itself as the group responsible for conserving and protecting fisheries resources, and with the organization's natural interest in maintaining its power. Increasing local autonomy means that DFO would, under Ministerial directive authorized by legislation, entrust some local management activities to one user group. Of course, that user group would have an interest in conserving and protecting the resource, a line of reasoning that underlies moves to local fisheries resources management worldwide (Ostrom 1990).[1]

Another related variable is the number of projects that are undertaken, given the fixed budget. At one extreme, the

[1]When developing strategies that involve delegation of authority, it is tempting to look at the comanagement experience elsewhere (e.g., northern Canada, the Pacific Northwest states) to see if examples can be drawn that serve as precedents for the British Columbia situation. These points are discussed in a companion paper (R.K. Paisley, M. Healey, and T. McDaniels, in preparation). However, it is important to note that the experience elsewhere may or may not be relevant in British Columbia. In some sense, searching for precedents is effectively a means of constraining the possible range of alternatives to be considered. Such constraints may be attractive from some viewpoints but not others. There is really no easy way to circumvent the difficult but crucial process of considering trade-offs among strategy alternatives.

TABLE 5. Variables under DFO control that are important for designing cooperative management strategies.

Variable	Range of options
1. Autonomy in selecting Native management projects	Low ⟷ high
2. Autonomy in reliance on local knowledge and resources in carrying out projects	Low ⟷ high
3. Degree of designed learning within projects	Low ⟷ high
4. Number of projects	Low ⟷ high
5. Average cost of projects	Low ⟷ high
6. Amount of spending by DFO on projects	Fixed by budget allocation
7. Allocation of fish to Native people	Not considered part of initial cooperative management concerns

resources could be widely distributed among a large number of small projects. At the other extreme, resources could be concentrated on a few large, significant projects. This variable also poses trade-offs for DFO. Given an objective to foster community involvement, one might decide on a large number of small projects. On the other hand, there are reasons to consider larger projects that have a greater chance of having meaningful (measurable) effects for fisheries management.

A final choice variable for DFO is in the degree of "active learning" designed into projects. "Active learning" means designing comanagement initiatives that provide adaptive management opportunities (Holling 1978). Learning opportunities could involve learning about comanagement mechanisms, as well as about fisheries concerns, such as stock dynamics. Here, trade-offs between "passive" and "active" learning strategies must be considered. In a passive approach, initial decisions would be made about such things as the degree of autonomy to be accorded First Nations groups, and then all projects would be designed to a similar level of autonomy. Any learning (in the sense of designed adaptive management experiments) from the implementation of such projects would be incidental. In an active approach, by contrast, some projects will be deliberately designed to test the trade-offs between autonomy and other variables and learning will be a primary consequence of such projects. Active adaptive designs are risky, but in a situation in which there is a limited number of local precedents on which to base procedures, they can provide the quickest way to discover effective approaches. Table 5 summarizes variables under DFO control in designing strategies and the range of possibilities for each variable. The table contains the elements in all possible strategies regarding salmon fisheries comanagement involving First Nations and DFO in British Columbia.

DFO has an equally complex array of options in terms of the administrative procedures for implementing cooperative management projects. One could imagine a scale of delegation of administrative authority that could be defined at four levels. At the lowest level, DFO would merely inform users about management regulations and enforce those regulations. This might be considered a realistic, if simplistic, description of the way fisheries management was conducted several decades ago. At the next level, DFO could explain its management proposals and seek advice and comment from users. DFO would then decide whether to modify its management in light of that advice. At this point on the scale, DFO is still setting the agenda and making decisions, but there are opportunities for users to influence those decisions. At the third level, DFO would work jointly with users to determine management strategies that either DFO or user groups then implement. At the fourth level, DFO would delegate full responsibility for management decision-making to users. This fourth stage might be viewed as similar to the delegation of administrative authority by Canada to British Columbia for management of resident freshwater fishes. It is important to recognize that DFO can only delegate authority under the auspices of the *Fisheries Act* and established administrative law precedent in Canada. It cannot simply delegate authority because of political pressure.

Generic Strategies

We used the contents of Tables 4 and 5 and the preceding discussions to create four generic strategies for cooperative management. As with any strategic planning process, a combination of judgment, analysis, available information, and creativity was used. The four strategies are as follows.

Incrementalist

Implement many projects that have a relatively low average dollar cost and involve specific tasks defined by DFO, little or no delegation of authority for decision making to the user group, and relatively limited opportunities for adaptive learning.

Dramatic change

Implement fewer projects that require higher capital investment but have a correspondingly higher potential pay-off, are selected with substantial input from First Nations people, have high delegation of authority for decision-making to users, and have a high component of active learning (i.e., the antithesis of the first strategy).

Delegation, limited experimentation

Implement projects that emphasize delegation of authority for decisions but have only a passive learning component.

Experimentation, limited delegation

Implement projects that involve limited delegations of authority but are highly structured by DFO to meet the learning objective.

Conceptually, each of these strategies has certain attractive features. The first reflects a classic incremental approach to policy adjustment. It is most easily encompassed within the existing bureaucratic structure, entails the smallest risks to the resource and to other fishing interests, and would be least threatening to managers who may mistrust the motivations and capability of users. Its principal drawbacks are that it is unlikely to satisfy the aspirations of First Nations groups and is unlikely to test the opportunities for

cooperative management. One might expect this approach to have a high probability of failure when tested against the objectives of cooperative management important from First Nations perspectives.

The second represents a whole-hearted embracing of the philosophy of cooperative management, and would be a radical departure from the status quo. Implementing this strategy in a comprehensive way would require decisions within DFO that could lead to reduced authority in carrying out its legislated mandate. It could also require protracted negotiations regarding project design criteria, a dispute resolution system, and ways to reduce opposition among other fishing interest groups.

The third and fourth represent less extreme approaches to testing the capability of cooperative management arrangements. They are framed in terms of delegation of authority and adaptive learning because these seem to be critical dimensions of cooperative management. The design of projects in terms of delegation of authority is critical because too little or too much delegation will detract from the need to foster trust and respect. To make progress toward a trusting and respectful relationship the affected parties need to use cooperative management to work jointly on problems. When either party retains too much control, communication barriers may persist and prejudices remain.

The design of projects in terms of adaptive learning is also critical because First Nations appear eager to take responsibility for resource management. The most efficient way to discover workable options is to design particular projects as experiments in process or technique. Active experimentation to speed adaptation is often difficult to implement in government agencies. However, in the case of the cooperative management program, standard operating procedures have not yet been established, and greater experimentation might be possible.

1992 First Nations Fishery on the Fraser River

The intent in outlining these comanagement strategy options was to represent the strategic trade-offs available to DFO among possible approaches for comanagement after 1991. In preparation for the 1992 season, DFO adopted an approach that is, in essence, our "Dramatic Change" strategy: a whole-hearted commitment to cooperative management with fewer projects, selected with considerable input from First Nations, with considerable delegation of authority and potential for adaptive learning.[2] Yet the implementation of the strategy was fraught with problems.

The experience in 1992 is highly instructive in terms of comanagement practice. The First Nations Fraser River sockeye salmon fishery was, at best, controversial (Pearse 1992) and was, at worst, viewed by some as a management disaster (*Vancouver Courier* 1993). The 1992 season provides substantial lessons about the implementation of comanagement strategies, about coping with change in a complex regulatory structure, and about the political aspects of First Nations fisheries.

Major changes occurred in preparation for the 1992 season that dramatically altered cooperative management and First Nations fisheries in British Columbia. On June 29, 1992, the Minister of DFO announced the new Aboriginal Fisheries Strategy. The general objectives of this 5-yr program are "to fulfil the government's newly defined obligation to consult with native people about how best to provide for their right to fish, and to end the conflict and litigation by engaging native people in managing fish resources and by providing them with new economic development opportunities" (Pearse 1992).

One substantial component of the strategy was the negotiation of some 80 formal interim agreements that assigned responsibilities for fisheries management, including regulating First Nations fishing to First Nations groups. These interim agreements form the second tier in the three-tiered approach to land claims negotiation mentioned in the introduction. The second, and perhaps even more sweeping, change was a series of special agreements with First Nations communities on the lower Fraser River and a few other locations, which, for the first time, provided specific negotiated allocations for the First Nations fishery and a 1-yr pilot project allowing for the sale of fish by First Nations people (Pearse 1992).

These steps appear to provide substantial progress in the evolution of cooperative management and in First Nations rights. They involved a negotiated framework, a selected set of projects, specified (short-term) rights to fish, delegation of authority for enforcement, and adaptive learning through a 1-yr experiment. Several factors combined to make implementation of these measures, and acceptance by the broader community of interested parties, extremely difficult. First, these agreements with First Nations were largely negotiated by the Deputy Minister of DFO and others from DFO headquarters. First Nations felt the need to negotiate directly with senior headquarters staff because regional officials were not perceived as committed to change (Pearse 1992). Commitment among regional staff, who had to make these new arrangements work, had not been sought or encouraged. Second, the agreements were negotiated piecemeal, with only some of the bands that traditionally fish Fraser River sockeye getting the authorization to sell their catch. First Nations bands in the upper Fraser River, who had not been given the opportunity to participate in the 1-yr experiment, perceived themselves as treated inequitably. Finally, other important stakeholder groups, particularly commercial and sport fisheries organizations, were not part of the process to develop these new arrangements (Pearse 1992).

Pearse (1992) discussed in detail the series of events in the First Nations 1992 fishery on the Fraser. Some 482 000 sockeye salmon seemed to "disappear" on their way to the spawning grounds, after being counted by hydroacoustic methods in the lower Fraser. Pearse's investigative report for the Minister of DFO estimates that some 200 000 fish were illegally caught in the upper reaches of the Fraser River, while another 280 000 may have died as a result of stress. Pearse's report on the issue is almost a catalogue of how not to conduct a cooperatively managed fishery: agreements were made at the eleventh hour, allowing virtually no time for planning; some groups

[2]Our work on strategy alternatives (which involved the earlier discussion in this paper) was submitted to DFO managers for First Nations programs in Vancouver in spring 1992. We cannot say to what extent our work played a role in development of the Aboriginal Fishing Strategy implemented in the summer 1992 season. We believe that the actual policy implemented was the result of extensive negotiation. This does not diminish the utility of viewing the overall process of developing objectives and strategies and the 1992 management outcomes as an adaptive management process.

felt excluded and thus had less responsibility for success; enforcement was almost nonexistent on upper reaches of the river; training and resources for First Nations guardians were insufficient; conflicts and neighbours; and there was a lack of communication about expectations, regulation, and conservation (Pearse 1992).

Despite the difficulties on the upper reaches of the river, there were some remarkable successes during the 1992 Fraser River First Nations fishery. The First Nations on the lower Fraser had signed agreements with DFO that gave these bands a specific allocation of fish, authorized sale of fish, and provided training and resources for enforcement (Pearse 1992). The "guardians" of the Lower Fraser Fishing Authority (from lower Fraser bands) were generally successful in enforcing local regulations in their area of responsibility. These bands took less than their assigned allocation of fish and the guardians helped ensure that those who fished had subsistence licenses and adhered to conservation rules (Crey 1993).

Discussion

Some of the lessons from the 1992 season are straightforward. Major changes in comanagement practice, particularly those involving turning over even minimal regulatory control to a regulatee, must be made carefully. There must be sufficient time to allow planning, good communication, and resources to ensure that the plans can be carried out. Excluding some participants without good reason is risky.

Some of the lessons of the 1992 season are more subtle. First, consider a point made earlier about the "Dramatic Change" strategy: it may be very difficult to implement this strategy rapidly, given the potential impacts on regional DFO managers and staff. This is not to suggest that regional DFO staff created the problem in the 1992 fishery through truculence. Rather, lack of resources and poor communication played a major role in the eventual breakdown of authority. A second point about this strategy, not anticipated before the 1992 season, was the potential for perceived inequities by First Nations groups not included in the "smaller number of projects" called for by the strategy. Where long-contested rights are involved, some bands may not be content to watch others experiment with new opportunities from which they are excluded.

A third point is the issue of fisheries allocation. We have noted that both the DFO representatives and the First Nations person stressed that allocation issues should not be part of cooperative management in the near term. The fear was that potential political problems and the possibility of resistance by other stakeholders were too great to risk losing the other benefits of comanagement such as building trust and cooperation. In hindsight, one might wonder if the move to permit sales of fish by First Nations in 1992 should have been delayed for at least one season.

A fourth point is drawn from the contrast between the experience on the lower Fraser, where First Nations management apparently worked well, and on the upper Fraser, where management and enforcement were nonexistent. The lower Fraser bands received an allocation of fish to sell, which gave them an incentive to participate and pay attention to the general conduct of the fishery, in hopes that the allocation would be renewed in future years. These bands also received resources to employ, train, and equip the First Nations guardians as enforcement officials, and responsibility to carry out enforcement in their area. Those who participated in the upper Fraser fishery received no allocation of fish to sell, and perceived that they were excluded from an important opportunity to earn money from fishing activities they consider their aboriginal right. Thus, they had a financial incentive to want to participate in the fishery, but little incentive to ensure that the fishery was conducted properly. They also had no incentive to govern the total catch, and little or no resources, training, and equipment for native enforcement. The lower Fraser bands had the benefit of all three of the policy levers available to DFO to create successful comanagement, while the upper Fraser participants had none. Seen in this light, the conduct of the upper Fraser fishery is not surprising.

A fifth point is the apparent inability of other fishing interests, particularly nonnative commercial and sport fishermen, to view the 1992 season as an experiment. One might reasonably expect some degree of illegal fishing to occur during the first season in which First Nations have the opportunity to sell their catch. Such difficulties might be expected in any major change in regulatory practice. Yet, other interested parties have been intolerant of the experimentation and learning that are necessary for adaptive approaches. For example, a broad-based coalition called the British Columbia Fishermen's Survival Task Force has repeatedly called for abandonment of the Aboriginal Fisheries Strategy (*Vancouver Courier* 1993). Comanagement appears to be an adaptive management context in which experiments should be carefully designed to avoid public, dramatic failures (Holling 1978).

A final point, which is a corollary to the previous ones, is that of timing. One of the essential tenets of organizational theory is that resistance to change is inevitable and can only be overcome slowly (Downs 1967). By adopting a somewhat slower pace for comanagement, with smaller steps to provide some initial successes, help built trust, and provide opportunities for learning, the controversial outcomes of the 1992 season could possibly have been avoided. A slower pace would have allowed more time for adjustment and a more open process involving other stakeholders, particularly commercial and sport fishermen. On the other hand, going slowly (in keeping with the incrementalist strategy) may not have been viewed as desirable by First Nations, unless the incrementalist approach had a clearly specified schedule for progress over time.

Conclusion

Taken together, the objectives, organizational issues, strategy alternatives, and management outcomes discussed in this paper can be viewed as one experiment in an adaptive process for design of salmon fisheries cooperative management initiatives. The process of clarifying objectives and constructing strategies provided a framework for thinking about how cooperative management could best be conducted after the 1991 season. The experience of the 1992 season indicated how one of the potentially attractive strategies could go awry in some areas and be successful in others. This paper serves as a "postaudit" of the 1992 regulatory experiment, providing feedback for adaptive improvement in cooperative management practice.

Some might wonder whether the results of the 1992 season call into question the benefits of clarifying objectives and strategies. Why bother with strategies when they can have

undesirable outcomes? Two answers are relevant. First, when there is so little experience with cooperative management, all parties should recognize the need to make an initial experimental attempt, and then review the results. Some approaches will inevitably work better than others, and repeated attempts with feedback provide the best opportunities for learning. A second point is that the results of the 1992 season can best be viewed as providing useful lessons in the importance of timing, attention to process, and attention to the details of implementation. These are pervasive stumbling blocks in developing new programs of all kinds, not just cooperative management (Williams 1989). However, the sharing of power that cooperative management entails makes these lessons particularly crucial for achieving long-term success.

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PERSPECTIVE

What Can Be Learned from the Collapse of a Renewable Resource? Atlantic Cod, *Gadus morhua*, of Newfoundland and Labrador

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Hutchings, J.A., and R.A. Myers. 1994. What can be learned from the collapse of a renewable resource? Atlantic cod, *Gadus morhua*, of Newfoundland and Labrador. Can. J. Fish. Aquat. Sci. 51: 2126–2146.

Temporal changes in demography, population sustainability, and harvest rates support the hypothesis that overexploitation precipitated the commercial extinction of northern cod, *Gadus morhua*, off Newfoundland and Labrador in 1992. Annual estimates of realized population growth (r) indicate that the stock was rarely sustainable at the age-specific survival and fecundity rates experienced since 1962. A twofold decline in annual survival probabilities in the 1980s was concomitant with increased inshore and offshore fishing effort, declining catch rate, and spatial shifts in gillnetting effort from areas of low (inshore) to high (offshore) catch rates. We reject hypotheses that attribute the collapse of northern cod to environmental change. Water temperature was associated neither with juvenile nor adult abundance nor with adult distribution by depth. Harvests equivalent to those of the past decade were sustainable in the nineteenth and early twentieth centuries in a considerably colder environment. An updated analysis of previous work indicates that salinity has little effect on recruitment. We conclude that the collapse of northern cod can be attributed solely to overexploitation and that population sustainability indices such as r provide a means by which the susceptibility and resilience of exploited populations can be assessed and their probability of commercial extinction reduced.

Les modifications temporelles des caractères démographiques, de la durabilité des populations et des taux de récolte soutiennent l'hypothèse selon laquelle la surexploitation a accéléré l'extinction commerciale de la morue du Nord, *Gadus morhua*, au large de Terre-Neuve et du Labrador en 1992. Les estimations annuelles de croissance réalisée des populations (r) indiquent que le stock était rarement durable aux taux de survie et de fécondité par âge qui ont existé depuis 1962. Une diminution deux fois plus importante des probabilités annuelles de survie dans les années 80 coïncidait avec une augmentation de la pêche côtière et hauturière, avec une diminution des taux de capture ainsi qu'avec les déplacements spatiaux de la pêche au filet maillant des zones à faible taux de capture (pêche côtière) vers des zones à fort taux de capture (pêche hauturière). Nous rejetons les hypothèses voulant que l'effondrement de la morue du Nord soit attribuable à des changements environnementaux. La température de l'eau n'était liée ni à l'abondance des juvéniles et des adultes ni à la distribution des adultes en fonction de la profondeur. Des récoltes équivalentes à celles des dix dernières années étaient compatibles avec la durabilité des populations au dix-neuvième siècle et au début du vingtième siècle dans un environnement considérablement plus froid. Une analyse récente des travaux antérieurs indique que la salinité n'a que peu d'effet sur le recrutement. Nous concluons que l'effondrement de la morue du Nord ne peut être attribué qu'à la surexploitation et que les indices du caractère durable des populations comme r constituent un moyen d'évaluer la sensibilité et la résilience des populations exploitées et de réduire la probabilité de leur extinction commerciale.

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Resource management is based on the premise that the existence of harvesting regulations better ensures the long-term viability of a population under commercial exploitation than does their absence. Ideally, such regulations should depend upon sound knowledge of the harvested organism's life history (i.e., age- and size-specific schedules of survival and fecundity), population structure (e.g., number and size of reproductively distinct populations within the geographical area of exploitation), and community ecology (i.e., the biological interactions that influence a species' abundance). In practice, the effort allocated to the collection of such biological data is limited. Instead, most management agencies concentrate on the formulation of indices of the past, present, and future states of the population (e.g., abundance, harvest-induced mortality), metrics assumed sufficient

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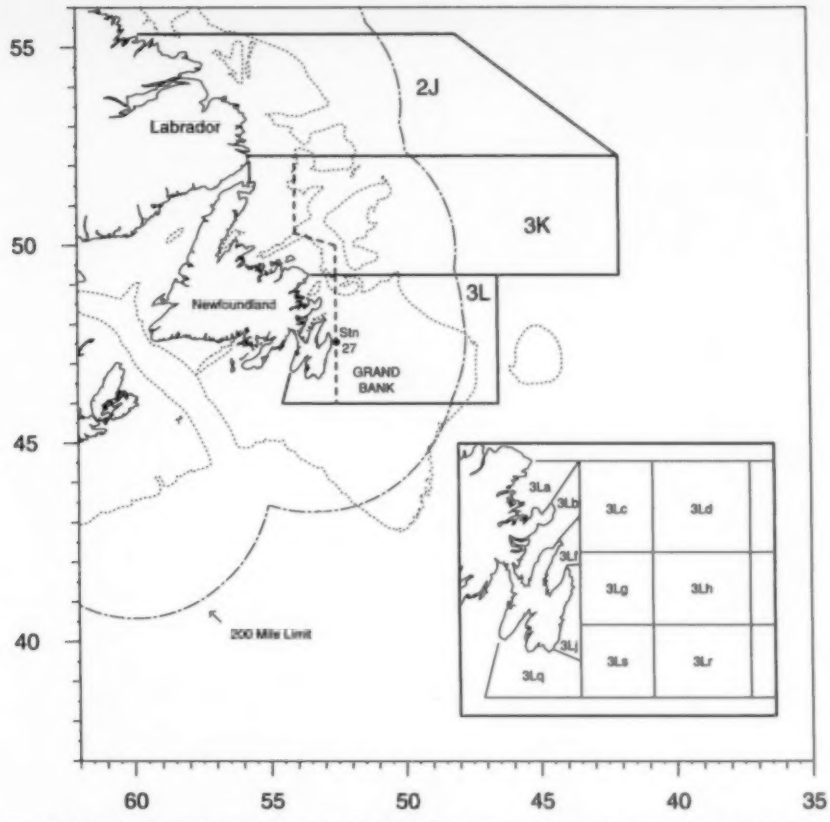

FIG. 1. Map of the study region. Cod inhabiting Northwest Atlantic Fishery Organization Divisions 2J, 3K, and 3L comprise the northern cod stock. Depth contour represents the 300-m isobath. The broken line separates the inshore and offshore jurisdictional unit areas in 3K and 3L (the latter are detailed in the inset).

to achieve long-term resource sustainability (Gulland 1983; Hilborn and Walters 1992).

The effectiveness of any management strategy can be difficult to assess. Despite the continued existence of a harvested population, it is impossible to determine whether a given management strategy is optimal because of the absence of a control (i.e., no management of a random sample of the same population) and the absence of other treatments (i.e., concurrent alternative management strategies on random replicates of the same population). It is only when a population declines in abundance to a level at which it is not economically viable for harvest (commercial extinction) and when its very existence is threatened that the reliability of a given strategy can potentially be evaluated. When a harvested population becomes commercially extinct, it is imperative that the potential causes of such biological devastation be identified and their relative contributions to the extinction evaluated (Beverton 1990; Ludwig et al. 1993; Rosenberg et al. 1993). Our objective is to determine the degree to which the commercial extinction of Atlantic cod, *Gadus morhua*, from the southern half of Labrador to northern Grand Bank of Newfoundland in 1992 was associated with temporal changes in water temperature, distribution by depth, and levels of exploitation (e.g., age-specific mortality, harvest rates, fishing effort, catch per unit effort).

Overview of the Northern Cod Fishery off Newfoundland and Labrador

Atlantic cod have been fished in Newfoundland waters since the late fifteenth century (Cell 1982). The fishery was largely a hook-and-line fishery until the development of cod traps in the 1860s and 1870s (de Loture 1949; Head 1976; Sinclair 1985). Major technological advances and increased efficiency in harvesting methods continued with the introduction of bottom trawlers in the early 1900s, particularly after the First World War (de Loture 1949), and nylon gill nets in the early 1960s (Templeman 1966). Annual landings of cod in Northwest Atlantic Fishery Organization (NAFO) Divisions 2J, 3K, and 3L (hereafter, northern cod; Fig. 1) increased from 100 000 to 150 000 t between 1805 and 1850, rose to more than 200 000 t during the latter half of the nineteenth century, and were frequently in excess of 250 000 t from 1900 to 1960 (ICNAF 1952; Harris 1990; Steele et al. 1992; Lear and Parsons 1993). With the advent of long-distance, "factory freezer", stern otter trawlers from Europe in the 1950s (Warner 1977), catches more than doubled from 360 000 t in 1959 to the historical maximum of 810 000 t in 1968. By 1977, when Canada extended its fisheries jurisdiction to 200 miles and most long-distance fishing was effectively eliminated, annual harvests had declined

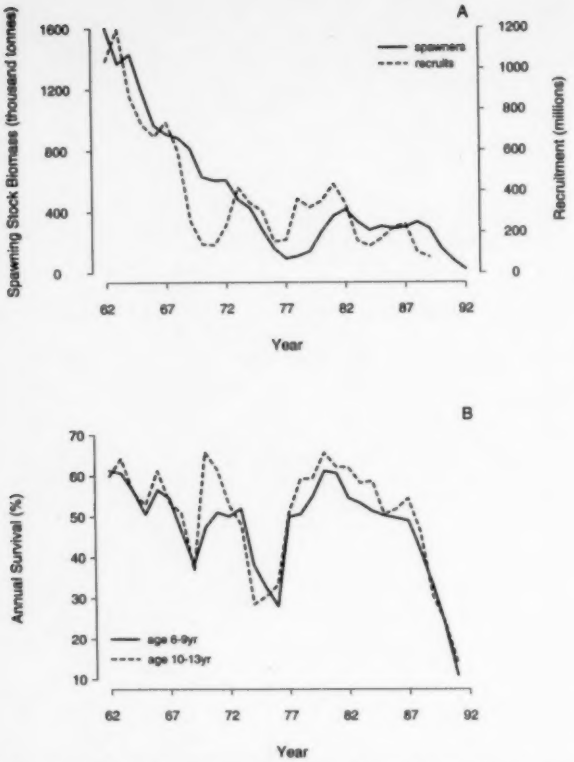

FIG. 2. (A) Variation in recruitment (numbers of 3-yr-old cod) and spawner biomass (individuals of ages 7 yr and older) of northern cod from 1962 to 1992 (data from Bishop et al. 1993b). (B) Annual survival (P) of mature northern cod aged 6–9 and 10–13 yr from 1962 to 1991. Fishing mortality (F) calculated from Baird et al. (1992) for 1962–77 and from Bishop et al. (1993b) for 1978–91; natural mortality is assumed to be constant at 18% per annum (i.e., $M = 0.2$, as defined by $P = e^{-(F+M)}$).

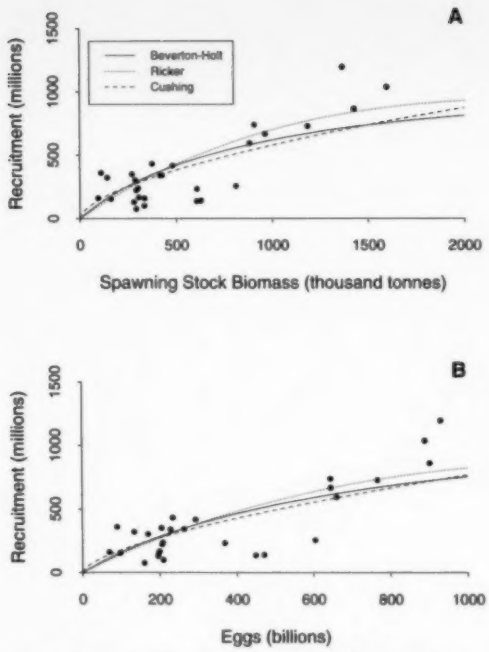

FIG. 3. (A) Three stock–recruit relationships for northern cod (Beverton–Holt, Ricker, Cushing) including recruitment year classes from 1962 to 1989. (B) Three fitted relationships (as in Fig. 3A) between recruitment and estimated number of eggs for recruitment year classes 1962–89.

almost 80% from the 1968 level (Bishop et al. 1993b). During the first 11 yr of Canadian management (1978–88), commercial landings of northern cod increased from 140 000 to 270 000 t. In 1991, 1 yr before the Canadian government imposed a moratorium on commercial exploitation of northern cod, landings were 64% less than those in 1988.

The number of harvestable northern cod (i.e., aged 3 yr and older) in 1992 was approximately 3 billion fewer than the number extant in the early 1960s (Baird et al. 1991a; Bishop et al. 1993b). The enormity of this decline can also be expressed through the reduction in biomass of the spawning portion of the population (approximated in assessment documents as individuals aged 7 yr and older) and in recruitment (abundance of 3-yr-old cod). Spawner biomass declined from an estimated 1.6 million t in 1962 (the first year for which estimates are available) to 22 000 t in 1992 (Fig. 2A) (Bishop et al. 1993b). This decline was associated with large fluctuations in annual survival probability (Fig. 2B). Annual survival probabilities for reproductive individuals declined from roughly 0.6 in the early 1960s to 0.3 in the mid-1970s and increased to 0.6 again in the early 1980s before declining to 0.1–0.2 in the early 1990s. The collapse of the spawning stock biomass had an equally large effect on the number of juveniles recruited to the fishery (Fig. 2A); recruitment is positively associated with spawner biomass in northern cod ($r^2 = 0.73$, $p = 0.0001$, p-value corrected for autocorrelations; Fig. 3A).

Hypotheses for the Collapse of Northern Cod

When the moratorium was announced, the collapse of northern cod was considered to be sudden, drastic, and unexpected and was attributed to increased natural mortality resulting from unusual environmental conditions, primarily in the form of cold water temperatures (Lear and Parsons 1993). A second hypothesis is that colder temperatures throughout the 1980s effected a southerly shift in the distribution of spawning individuals during the 1980s that has resulted in the rearing of juveniles in suboptimal habitat (deYoung and Rose 1993). If true, we would expect a negative correlation to exist between cod abundance (adults and recruits to the fishery) and sea temperature. It has also been suggested that cod have moved to deeper water in recent years (Bishop et al. 1993b). A short-term, large-scale emigration to deeper water in the early 1990s would be evident from a temporal analysis of cod biomass in deep water relative to that in shallower water. Given that total stock failure has not been previously recorded for northern cod within the past two centuries, these hypotheses make the implicit assumption that environmental conditions experienced during the early 1990s, or the 1980s, were temporally anomalous.

These hypotheses assume either explicitly or implicitly that an environmental variable is a primary determinant of cod survival and that the dramatic reduction in annual survival probabilities throughout the 1980s and early 1990s (Fig. 2B) cannot be wholly explained by increases in fishing mortality. When the moratorium on northern cod was announced on 2 July 1992, it was noted that "the increase in fishing mortality in...recent years is inconsistent with the trends in fishing effort by Canadian fleets. The total number of hours fished by the Canadian otter trawl fleet in Div. 2J+3KL declined consistently from 63,000 [h] in 1988 to 43,000 [h] in 1991" (NAFO 1992, p. 26). This assertion was repeated in the Canadian Fisheries Resource Conservation Council's "1993 Report on the Status of Groundfish Stocks in the Northwest Atlantic" (FRCC 1993). But the implication that overfishing was not the primary contributing factor responsible for the decline of northern cod can be assessed neither in the absence of information on fishing effort *prior to 1988* (by offshore *and* inshore gear) nor in the absence of temporal data on population sustainability.

We wish to examine competing hypotheses for the collapse of northern cod by addressing the following questions: (1) Were water temperatures experienced by northern cod in the 1980s and early 1990s temporally anomalous? (2) Does cod mortality increase with decreasing water temperature? (3) Did cod move to deeper waters in the early 1990s? If so, can such a displacement be attributed to colder temperatures? (4) Was the temporal increase in total mortality throughout the 1980s inconsistent with observed temporal and spatial patterns in inshore and offshore fishing effort and catch rate? (5) Were observed age-specific rates of survival and fecundity sufficient to sustain the population through time?

We begin with an analysis of depth-stratified temperature data that have been collected approximately twice monthly at a single location since 1946 (Station 27, ranging in depth to 175 m, is located immediately east of St. John's; Fig. 1) as a proxy for interannual variation in water temperature throughout 2J3KL (Petrie et al. 1992). We then examine historical associations among continental shelf water temperatures (from 1910), ice clearance data (from 1803), air temperatures (from 1874), and commercial cod landings (from 1805) to provide a broad perspective of the environmental conditions in which the northern cod fishery was sustainable during the nineteenth and twentieth centuries. The relative importance of water temperature on cod population biology is assessed by statistically analyzing associations between temperature and (1) adult abundance, (2) juvenile abundance, and (3) depth distribution of cod. Research survey data are analyzed to examine temporal variation in the biomass of cod in deep water relative to that in shallow water. We analyzed NAFO fishery statistics to assess the degree to which annual increases in total mortality throughout the 1980s and early 1990s were associated (1) with yearly changes in fishing effort and catch rate by Canadian trawlers and (2) with spatial and temporal variation in the inshore and offshore gillnet fisheries. Demographic changes in northern cod are described by changes in age distribution of reproductive individuals, weight-at-age, and age-specific rates of survival and fecundity. Estimates of the latter two parameters were used to estimate r, a measure of population growth, to identify those years from 1962 to 1992 in which northern cod were able to sustain themselves, i.e., when $r \geq 0$.

Materials and Methods

Historical Temperature and Commercial Catch Data

Depth-stratified water temperature data have been recorded approximately twice monthly since 1946 at Station 27 (Fig. 1). Data are generally available at 10-m depth intervals from the surface to 50 m and at every 25 m thereafter to the bottom at 175 m. Although small-scale, short-term temperature fluctuations in 2J3KL may not be represented by these data, they do reflect large-scale variability in temperature and other environmental indices (e.g., areal ice cover, cold intermediate layer (CIL, volume of <0°C water), North Atlantic Oscillation (NOA) sea level pressure anomaly) throughout the region (Petrie et al. 1992; Colbourne et al. 1994). To remove seasonal effects on temperature, we report these data as anomalies from the long-term mean (1946–92). For analyses of adult abundance, we excluded temperatures recorded at less than 50 m, depths which demersal mature cod are unlikely to inhabit and from where abundance data are not available. Analyses involving juvenile abundance (i.e., recruitment) included temperatures recorded from 0 to 50 m (depths inhabited by larval cod; Anderson and deYoung 1993). Depth-averaged water temperature anomalies were calculated for each year using the formula

$$(1) \quad \sum_{i=1}^{N-1}((x_i + x_{i+1})/2)(d_{i+1} - d_i)$$

where x_i represents the yearly average temperature anomalies at depth strata $i,\ldots,N$ and d_i represents the depth of stratum i. This calculation weights the mean temperature between successive strata by the difference in depth between strata. The p-values of all correlation coefficients between temperature and abundance were corrected for autocorrelation.

Long-term data on water temperatures in 3L (i.e., northern Grand Bank), dates of ice clearance, and air temperature in the region are available from 1910, 1803, and 1874, respectively. Shelf water temperatures in 3L (100 m depth) were obtained from the MEDS (Marine Environment Data Services, Ottawa, Ont., Canada) data base. Using observational data from the Labrador coast, Newell (1991, 1992, 1993) calculated an ice clearance index which reflected the day of year on which the waters off Hopedale, Labrador (55°05′N), were clear of Arctic pack ice. We report these data as departures from the long-term (1800–1993) mean. The ice clearance index is correlated with depth-averaged (50–175 m) water temperature at Station 27 ($r = 0.50$, $p = 0.0004$, $n = 46$). Air temperatures have been recorded at St. John's, eastern Newfoundland (15 km from Station 27), since 1874 and are available from the Canadian Atmospheric and Environment Service. We report these data as anomalies from the long-term (1874–1992) mean. Depth-averaged water temperature at Station 27 is correlated with St. John's air temperature ($r = 0.48$, $p = 0.0006$, $n = 46$). Historical commercial catch records were derived from those estimated for northern cod by Hutchings and Myers (1995) and based upon data collated by Gosling (1910), ICNAF (1952), Harris (1990), Bishop et al. (1993b), and W.H. Lear (Department of Fisheries and Oceans, Biological Sciences Directorate, 200 Kent St., Ottawa, ON K1A 0E6, Canada, unpublished data).

Ice clearance and air temperature data were smoothed using a robust locally weighted regression, *loess*, a method recommended for scatterplot data with missing values (Cleveland 1979; Hastie and Tibshirani 1990). The value at x_k fitted by the smoothing procedure is the value of a polynomial fit to the data using weighted least squares. The weight given to (x_i, y_i) is large if x_i is close to x_k and small if it is not.

Research Survey Abundance and Biomass Data

Recent temporal changes in the abundance and biomass of adult cod were obtained from research surveys conducted in autumn by the Canadian Department of Fisheries and Oceans from 1978 to 1992 in 2J and 3K and from 1981 to 1992 in 3L. Survey estimates of abundance and biomass are based upon the actual number and biomass of cod captured in a series of tows of a bottom otter trawl within each of several depth-stratified sampling strata within each NAFO division. There are generally 5–30 sets per stratum and there are 24, 23, and 29 strata within Divisions 2J, 3K, and 3L, respectively. The number (or biomass) of cod in division i is simply the sum of the numbers of cod estimated for all strata j in that division:

$$(2) \quad N_i = \sum_{j=1}^{s} N_j .$$

The number of cod in stratum j, N_j, is estimated as the mean number of cod captured per tow in stratum j (summed over trawls $t = 1...x$) increased by a proportion equal to the area of stratum j, A_j, divided by the total area sampled by the trawls, i.e.,

$$(3) \quad N_j = \bar{N}_j \left(A_j / \sum_{t=1}^{x} A_t \right).$$

Abundance data were also obtained from depth-stratified random research surveys conducted by German vessels from 1972 to 1983 in 2J (Messtorff 1984). Their survey designs are comparable with the later Canadian surveys. German researchers also employed 30-min tows of otter trawls and conducted their surveys during late autumn in the same strata sampled by the Canadian vessels from 1978 to 1992.

VPA Estimates of Abundance and Biomass

Unless identified as being based solely upon research vessel survey data, indices of cod abundance and biomass were those determined from a virtual population analysis (VPA) conducted using a process called ADAPT (described by Mohn and Cook 1993). For northern cod, this procedure combines age-specific indices of population size from both commercial and research vessels, or more recently from research vessels only, to estimate historical stock sizes from 1962 to the present (Gavaris 1988; Conser 1993). The age-specific groups of cod referred to in the text for which abundance or biomass was estimated from the VPA are *recruits* or *recruitment* to the fishery (abundance of cod of age 3 yr), *spawner* or *spawning stock biomass* (cod of age 7 yr and older), and *harvestable biomass* or *abundance* (cod of age 3 yr and older).

Distribution by Depth of Cod Biomass

Cod biomass was estimated from the autumn research surveys conducted in 2J3KL (data in Bishop et al. 1993b). Temporal changes in biomass were documented for the four main depth classes in each region in which surveys were conducted. These depth classes ranged from 100 to 500 m, in increments of 100 m in 2J and 3K, and 57–92, 93–183, 184–275, and 276–366 m in 3L. Associations between water temperature and cod biomass in deep water relative to that in shallow water were determined by calculating correlation coefficients between depth-averaged Station 27 temperature anomalies and two indices of deepwater cod biomass. The latter were biomass between 300 and 500 m relative to that between 100 and 300 m in 2J and 3K and biomass between 184 and 366 m relative to that between 57 and 184 m in 3L.

Catch and Effort Data

Commercial catch and effort data on Canadian offshore trawlers were obtained from fishery statistics compiled by NAFO from 1979 to 1991 (1979–90 data are from NAFO (1984–94); 1991 data are available from NAFO although they have not yet been published). These statistical summaries report the catch of all species caught in each NAFO division. Catches are further subdivided by country of origin, by gear type, by vessel tonnage, and by the target species being fished. We restricted our analyses to vessels for which cod constituted the largest biomass of species caught. From 1978 to 1991, nominal effort data were available for 87–100% of the annual cod biomass caught by otter trawls in 2J, 82–100% in 3K, and 91–100% in 3L. Most of the harvested cod was caught by vessels fishing primarily for cod (as percentages of the annual harvested cod biomass: 85–100% in 2J, 86–94% in 3K, and 64–88% in 3L). From 1981 to 1991, Canadian trawlers accounted for an average 52% of the Canadian catch and 44% of the total catch of northern cod (calculated from Bishop et al. 1993b).

Catch and effort data were also analyzed for cod captured by traps and gill nets. The data were compiled by STACAC (Statistical Co-ordinating Committee for the Atlantic Coast, Department of Fisheries and Oceans, Ottawa, Ont., Canada) and are available from 1985 to 1992. These data report catches on a considerably smaller spatial scale than those in the aforementioned NAFO records; data are summarized for 14 unit areas in 2J, 11 in 3K, and 14 in 3L. Effort is reported as number of trap days and number of clearances per 100-m gill net.

Population Sustainability

The instantaneous rate of change of the northern cod stock was estimated as r and given by the discrete-time version of the Euler–Lotka equation

$$(4) \quad 1 = \sum l_x m_x e^{-rx},$$

where l_x represents survival from the zygote to the beginning of the breeding season at age x and m_x is the number of female zygotes produced by a female breeding at age x (Cole 1954; Schaffer 1974). r is frequently used as a measure of fitness in evolutionary ecology (Charlesworth 1980; Stearns 1992; Hutchings 1993). As a measure of the rate of change in population abundance, population size at time $t + 1$ can be estimated from population size at time t from the equation $N_{t+1} = N_t e^r$ (Caughley 1977; Roff 1992). Thus, $r = 0$ for a population that is neither increasing nor decreasing, i.e., sustainable through time.

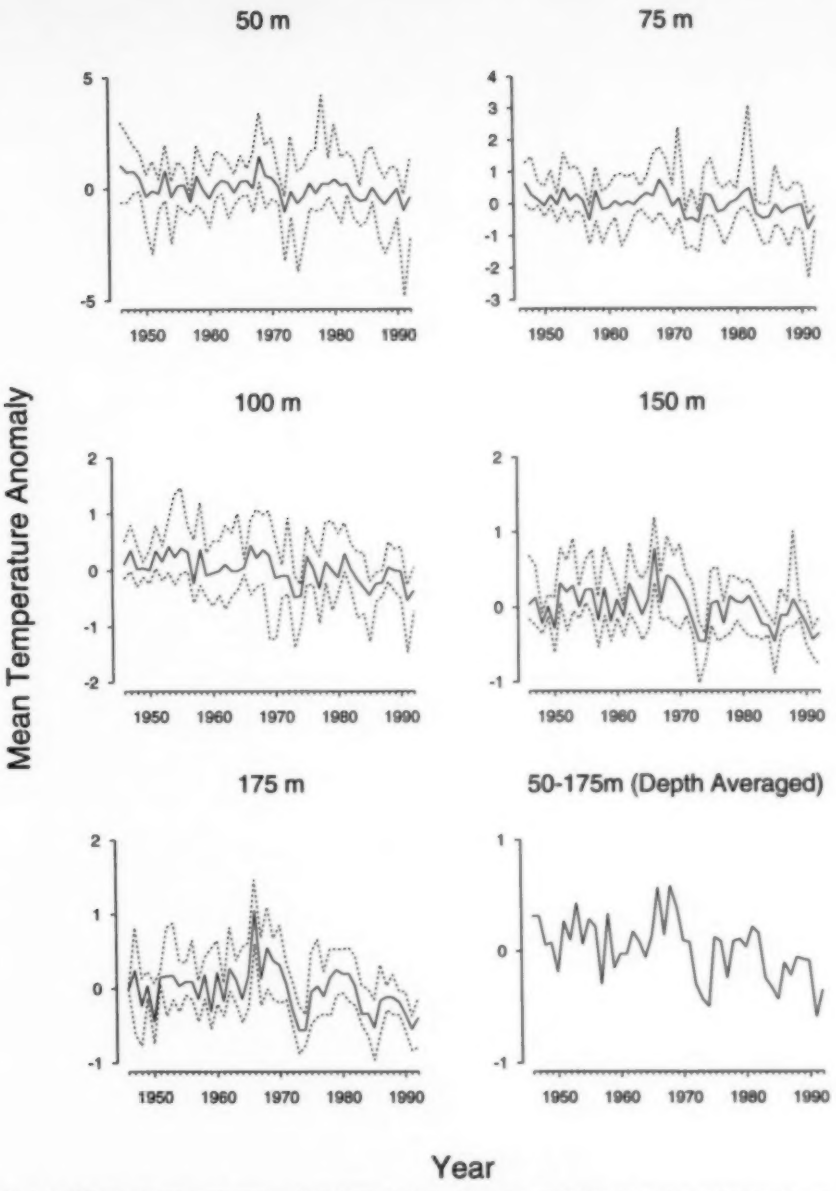

FIG. 4. Water temperature anomalies from the long-term mean (1946–92) at Station 27, immediately east of St. John's, Newfoundland, at depths of 50, 75, 100, 150, and 175 m and averaged across depths between 50 and 175 m. Solid lines represent annual temperature anomalies; broken lines indicate the maximum and minimum monthly temperature anomalies for each year.

We included age-specific rates of survival, l_x, and fecundity, m_x, for northern cod from 1962 to 1992. Fishing mortality estimates for ages 3–13 yr are available in Baird et al. (1991a) for the 1962–77 period and from Bishop et al. (1993b) for the 1978–92 period. Natural mortality per annum for all ages was assumed to be 18% (Baird et al. 1992; Bishop et al. 1993b). To allow for estimation errors in fishing mortality, F, we calculated r at F-values 30% greater and less than those provided in the assessment documents (Baird et al. 1991a; Bishop et al. 1993b). For comparison, an independent review of the northern cod stock suggested an error rate of ±22% for the overall fishing mortality rate estimated for 1988 (Harris 1990).

Age-specific fecundities were estimated from the fecundity versus weight regressions calculated by May (1967). Weight-at-age data from the commercial fishery are available from 1972 to 1992 (Bishop et al. 1993b). Data from 1962 to 1971 were approximated by the average weight-at-age from 1972

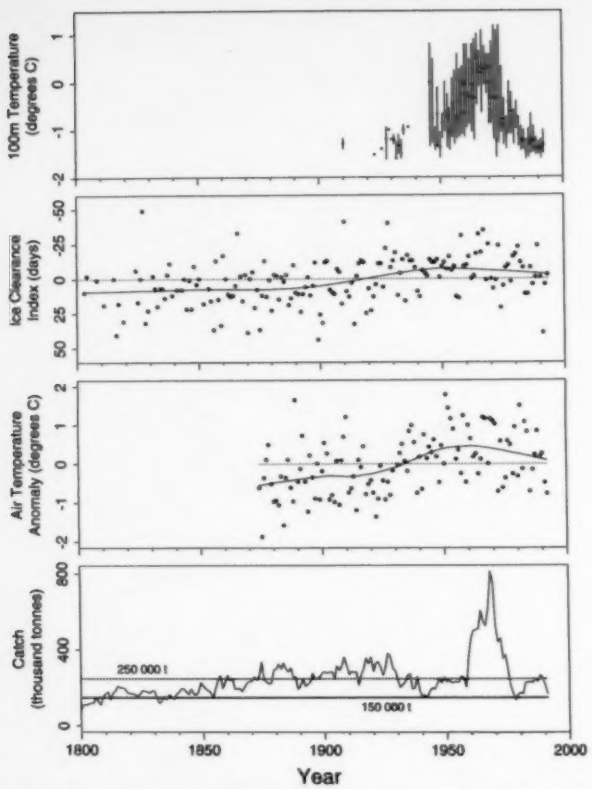

FIG. 5. Temporal variation in water temperatures on the continental shelf in 3L, date of ice clearance, air temperature, and commercial catch of Atlantic cod in NAFO Divisions 2J3KL. Shelf temperatures in 3L at 100 m (1910–92; MEDS data base) are represented by the median with the upper and lower quartiles represented by the lines above and below each median, respectively. Ice clearance index (1800–1993) represents the departure in days from the 1964–84 median clearing date at Hopedale, Labrador (data from Newell 1991). Air temperature anomalies from the long-term mean (1874–1992) recorded by Canadian Atmospheric and Environment Service at St. John's, Newfoundland. Estimated commercial catch of Atlantic cod from the region corresponding to 2J3KL from 1800 to 1992 (data from Hutchings and Myers 1995).

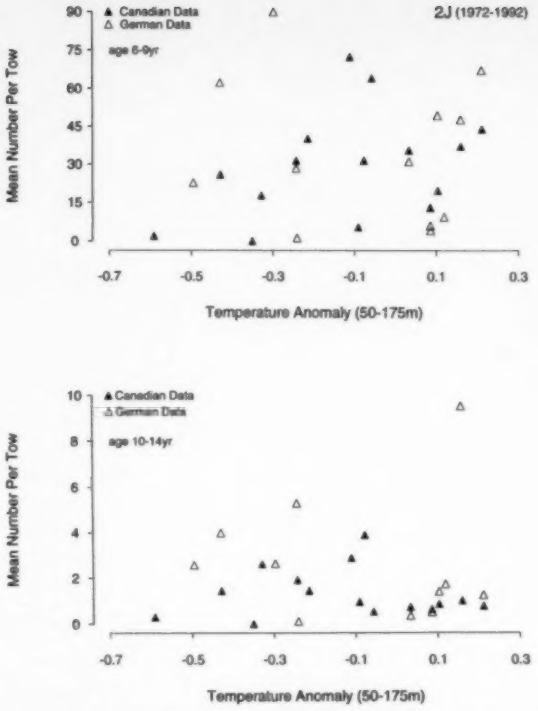

FIG. 6. Scatterplots of mature cod abundance against Station 27 water temperature anomaly in NAFO Division 2J. Data are from autumn research surveys conducted by German and Canadian vessels from 1972 to 1992.

to 1992. The similarity between the 1972–92 averaged weights-at-age and those estimated from autumn sampling by Canadian research vessels in 2J and 3K in the early 1950s and early 1960s suggests that such an approximation is a reasonable one (unpublished data). Based upon empirical data available from 1973 to 1992 (Shelton and Morgan 1993), we assumed that 0% of females reproduce prior to age 5 yr, 10% at age 5, 50% at age 6, and 100% at age 7 and at all subsequent ages until death following reproduction at age 15 (fish older than 15 yr constituted a median 1% of the total harvested biomass from 1962 to 1991; Bishop et al. 1993b).

To estimate survival from birth to age 3 yr (ages for which we do not have data), we plotted recruitment (number of 3-yr-old cod) against the expected fecundity contribution of the reproductive portion of the population (i.e., the summed product of age-specific fecundities and age-specific abundances). Three stock–recruit relationships were applied to the data (Fig. 3B): the Ricker, Beverton–Holt, and Cushing models (described by Cushing (1971) and Hilborn and Walters (1992)). Survival from birth to age 3 was estimated from the Ricker and Cushing models which generally encompassed the stock and recruit values predicted by the Beverton–Holt model. By using these models, the influence of density on juvenile survival (Myers and Cadigan 1993) is explicitly included in our estimates of sustainability.

Results

Interannual Variation in Water Temperature

Significant interannual variation exists among the 47 consecutive years of water temperature data at Station 27 in 3L ($F_{[46,463]} = 12.17$, $p = 0.0001$; Fig. 4). The time series of depth-averaged data indicates that temperatures were below the long-term mean in the early 1970s, the mid-1980s, and the early 1990s (lower right panel in Fig. 4). These relatively cold years (1972–74, 1984–85, and 1991–92) are evident within each depth interval and did not differ in temperature ($F_{[6,73]} = 1.94$, $p = 0.09$; depth-averaged temperatures from 20 to 175 m did not differ among these years either ($F_{[6,73]} = 1.91$, $p = 0.09$)). Thus, as measured by annual, depth-averaged temperature anomalies, the environmental conditions of 1991 and 1992 were also experienced from 1972 to 1974 and during 1984 and 1985. Minimum monthly anomalies at 50 and 75 m were lowest in 1991. However, those at 100, 150, and 175 m in 1991 were similar to or

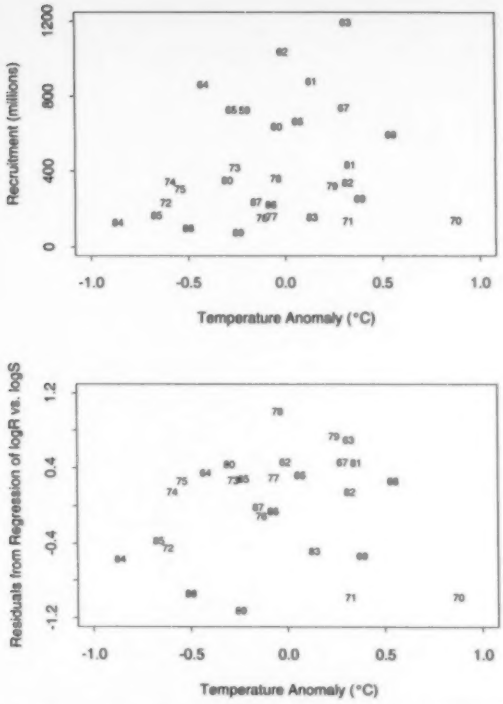

FIG. 7. Scatterplots of two indices of recruitment to the northern cod fishery against Station 27 water temperature anomaly (depth-averaged from 0 to 50 m). Upper panel: dependent variable represents millions of cod aged 3 yrs. Lower panel: dependent variable represents residuals from the linear regression of $\log_{10}$(recruitment) versus $\log_{10}$(spawning stock biomass). Data are indicated by year class.

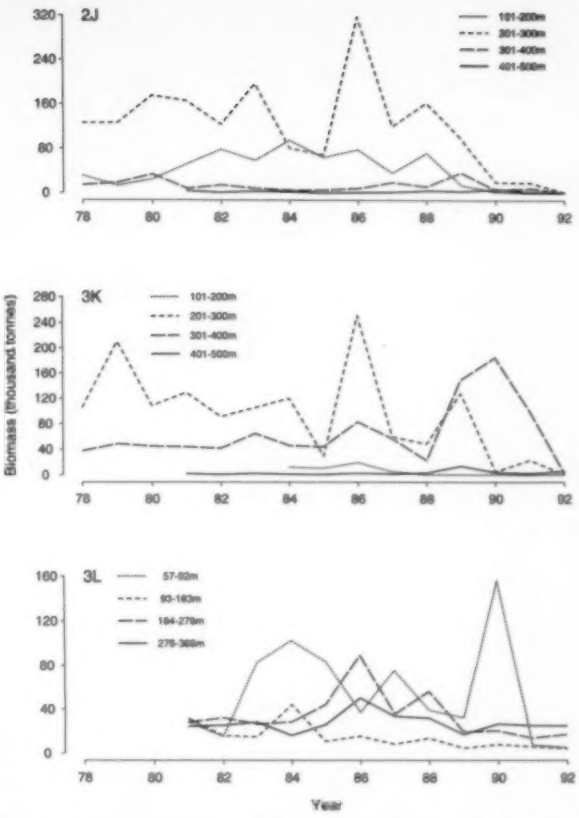

FIG. 8. Temporal changes in cod biomass at four depth intervals for which extensive sampling was conducted during autumn research surveys in NAFO Divisions 2J, 3K, and 3L. Standard errors of the annual total biomass estimates (all depths included) averaged ±37% in 2J, ±41% in 3K, and ±16% in 3L (data from Bishop et al. 1993b).

less than those experienced in the early 1970s, the mid-1980s, and, for the 175 m depth, the late 1940s.

There was no indication that cod landings throughout the nineteenth century were deleteriously affected on the scale of the major fishery collapse experienced in 1991 despite environmental conditions colder than those of recent years (Fig. 5). Shelf water temperatures in 3L similar to or less than those of the early 1990s were experienced in the 1920s, 1930s, and late 1940s when commercial landings averaged roughly 250 000 t. Dates of ice clearance from the Labrador coast and air temperature at St. John's have been considerably earlier and warmer, respectively, in the past several decades than they were throughout the nineteenth and early twentieth centuries when northern cod landings ranged from 100 000 to 300 000 t. Thus, landings that were not sustainable in the 1980s were sustainable in colder environments during the nineteenth and early twentieth centuries.

Water Temperature and Cod Abundance

The German and Canadian surveys conducted over 21 yr (1972–92) in 2J constitute the longest continuous time series of research abundance estimates available for northern cod. Scatterplots of abundance (combined data sets) versus temperature anomaly indicate that abundance of mature cod during this period was not associated with water temperature (Fig. 6; cod aged 6–9 yr: $r = 0.08$, $p = 0.72$; cod aged 10–14 yr: $r = -0.03$, $p = 0.89$). The absence of significant associations persists if the German and Canadian data are examined separately (cod aged 6–9 yr: $r_{CAN} = 0.39$ ($p = 0.15$), $r_{GER} = -0.13$ ($p = 0.68$); cod aged 10–14 yr: $r_{CAN} = -0.04$ ($p = 0.89$), $r_{GER} = -0.08$ ($p = 0.80$).

The association between water temperature and juvenile cod abundance was examined by plotting numbers of recruits to the fishery (i.e., cod aged 3 yr) against the depth-averaged temperature anomalies recorded at Station 27 for the upper 50 m of the water column (depths at which cod eggs and larvae are found on the Newfoundland Shelf; Anderson and deYoung 1993) (Fig. 7, upper panel). The correlation coefficient between temperature and recruitment is not significant ($r = 0.19$, $p = 0.54$). When the effect of spawning stock size on recruitment is removed from the analysis, there is still no evidence that recruitment is associated with any range of water temperature during the first year of life ($r = 0.08$, $p = 0.78$; Fig. 7, lower panel). Nor are there significant associations between recruitment (effects of spawner stock removed) and depth-averaged temperature anomalies in the upper 10 m ($r = -0.14$, $p = 0.58$), upper 20 m ($r = -0.09$, $p = 0.74$), or the entire water column from 0 to 175 m ($r = 0.18$, $p = 0.47$). Similar nonsignificant associations exist between recruitment and temperature experienced during

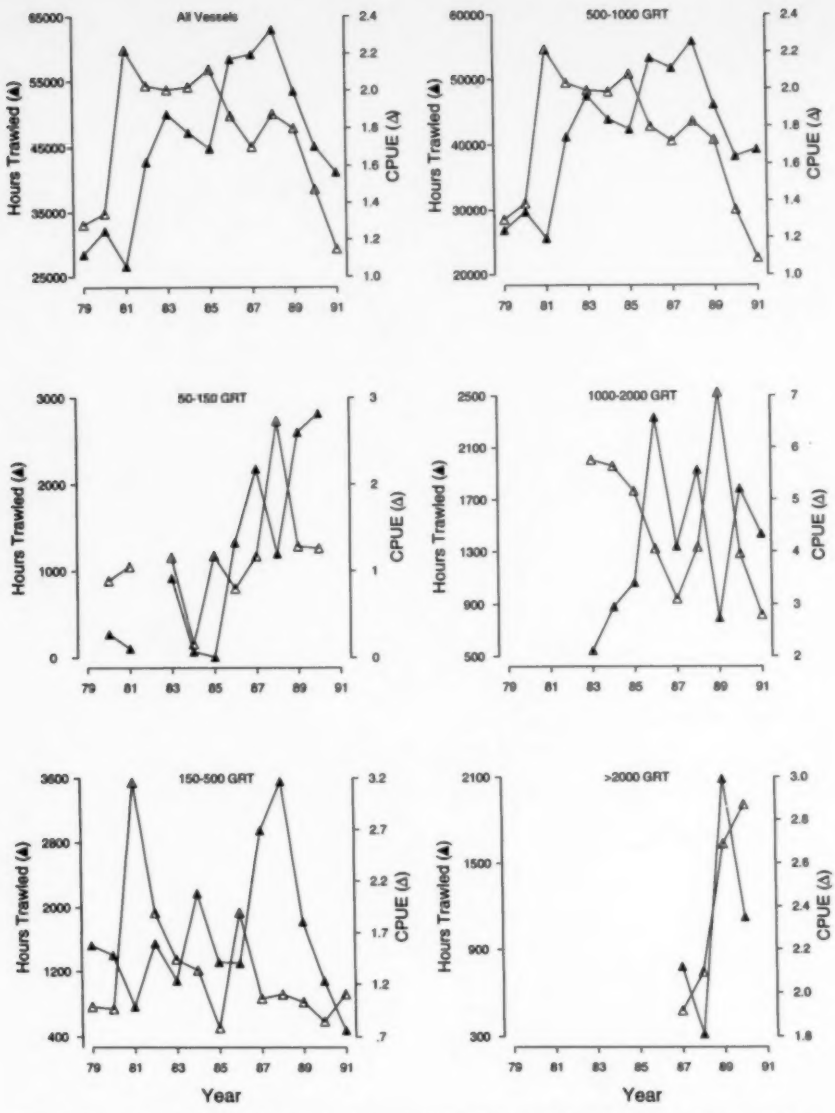

FIG. 9. Temporal variation in nominal commercial fishing effort (hours trawled; solid triangles) and catch per unit effort (CPUE, metric tonnes of cod per trawling hour; open triangles) for Canadian otter trawlers fishing for Atlantic cod in NAFO Divisions 2J3KL from 1979 to 1991 (GRT = gross registered tonnage).

the second and third years of life (upper 50 m of water, $r = 0.23$, $p = 0.40$ and $r = 0.46$, $p = 0.08$, respectively).

Interannual Variation in Depth Distribution of Cod

The biomass of northern cod in deep water, relative to that in shallow water, has not been significantly greater in the 1990s than in previous years and there is no evidence that the depth distribution of cod is related to water temperature. Any apparent proportional increase in cod biomass in deep water is due to the near absence of fish in shallow water (Fig. 8). In 2J, cod biomass from 100 to 300 m has declined by at least two orders of magnitude since 1978 whereas biomass between 300 and 500 m has changed little through time. Biomass at depths 300–400 m in 3K increased between 1989 and 1991 whereas that between 400 and 500 m was consistently low. Shallow water (100–300 m) biomass in 3K declined almost 100-fold over the 15-yr period. In 3L, cod biomass in deep water (184–366 m) in the early 1990s was lower than or similar to estimates from 1981 to 1989. As with the other two regions, biomass in shallow water (57–183 m) in 3L declined by almost two orders of magnitude through the 1980s and early 1990s. Water temperature was not significantly associated with deepwater cod biomass (>300 m in 2J and 3K, >184 m in 3L), relative to biomass in shallower water, in any of the regions (2J: $r = -0.35$, $p = 0.27$; 3K: $r = -0.28$, $p = -0.47$; 3L: $r = -0.08$, $p = 0.80$).

Temporal Trends in Fishing Effort and Nominal Catch per Unit Effort of Canadian Trawlers

The harvesting pressure imposed by offshore trawlers on northern cod was assessed by examining temporal changes in fishing effort (number of trawling hours by Canadian vessels) and catch per unit effort (CPUE, expressed as tonnes of cod captured per hour trawling) (Fig. 9). Catch and effort data are presented for all vessels and for five of the six tonnage classes of Canadian stern trawlers separately (the smallest tonnage class, not shown here, caught less than 1% of the annual trawler harvest from 1979 to 1991). Annually, more than 90% of the cod biomass caught by Canadian trawlers was harvested by vessels between 500 and 1000 gross registered tonnage (GRT, upper right panel of Fig. 9). From 1981 to 1988, fishing effort for these vessels more than doubled from 25 544 to 55 923 trawling hours whereas CPUE declined 23% over the same time period. Hours trawled and CPUE were negatively correlated for the 500–1000 GRT vessels ($r = -0.90$, $p = 0.01$, p-value corrected for autocorrelation) and for all vessels combined ($r = -0.89$, $p = 0.02$). The 54% increase in trawling hours between 1981 and 1991 for 500–1000 GRT vessels was associated with a halving of CPUE. Negative associations between effort and CPUE are also evident for 150–500 GRT vessels and among most years for 1000–2000 GRT vessels. It is noteworthy that during the first 4 yr of operation of the latter vessels, whose harvesting efficiency was generally more than double that of other vessels, a 30% decline in CPUE was associated with an almost fivefold increase in effort.

Temporal Trends in Gillnet Catches and Nominal CPUE

Gillnet landings of cod throughout 2J3KL increased from 11 435 to 47 000 t between 1974 and 1982, declined rapidly between 1982 and 1985 to 20 500 t, and increased again to almost 47 000 t in 1989 before decreasing to 17 300 t in 1991 (Fig. 10A). Temporal variation in gillnet catches in 2J3KL is reflected largely by catches in 3L. Inshore landings in 3L increased from 19 500 to 31 000 t in 1987 and remained at that level for two more years before declining by 50% in each of 1990 and 1991 (Fig. 10B). Offshore gillnet catches increased dramatically from 8 t in 1985 to 19 448 t in 1990, comprising 45 and 71% of the total gillnet catch in 2J3KL in 1990 and 1991, respectively. The dramatic spatial changes that occurred during the late 1980s in the fixed-gear fishery are well illustrated by the observation that between 1985 and 1990, offshore gillnet catches of northern cod increased from less than 1 to 18% of the total fixed-gear landings of northern cod.

Inshore trap and gillnet catches were associated with declining catch rates throughout the latter half of the 1980s (Fig. 11). Catch rates in the 3K and 3L trap fisheries decreased from 1986 to 1991 with the decline clearly evident by 1989. Notably, a tripling of trap catches in 3L between 1987 and 1990 was associated with a 70% reduction in catch rate. Significant reductions in gillnet catch rate were evident in Trinity Bay by 1988, Bonavista and Conception bays by 1989, and between St. Mary's Bay and the Southern Shore by 1990. Stable or declining gillnet catches were associated with declines in catch rate of 80, 60, and 76% in Bonavista (1986–89), Conception (1987–89), and Trinity (1985–89) bays, respectively. Between 1986 and 1990, catches from

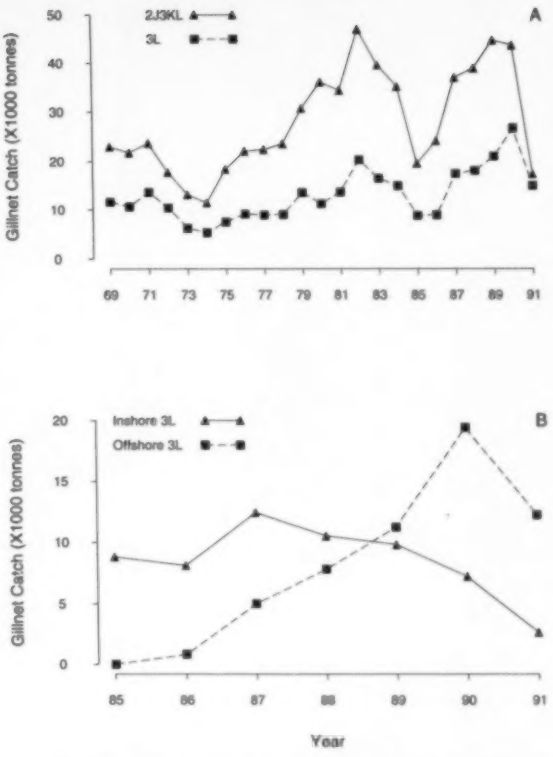

FIG. 10. Temporal variation in gillnet catches of northern cod in (A) 2J3KL and 3L and (B) inshore and offshore unit areas (defined in text) in 3L.

St. Mary's Bay and along the Southern Shore declined 21% whereas catch rate declined 30%.

Offshore harvest of cod by gill nets began after the sharp decline in inshore gillnet landings in 2J3KL between 1982 and 1985 (Fig. 10B). The majority of offshore cod captured by gill net were harvested in the Virgin Rocks area in central Grand Bank (unit area 3Lr) (Fig. 1 and 12). The increase in catch from Virgin Rocks from nil in 1985 to 17 257 t in 1990 was associated with a 53% reduction in catch rate. This pattern of rapid decline from initially high catch rates that exceeded those experienced inshore to relatively low rates was evident throughout the Northeast Newfoundland Shelf (unit areas 3Kbcefg) and throughout north and central Grand Bank (Fig. 12).

Temporal Changes in Age-Specific Weight and Abundance

Temporal changes in age-specific body weight are evident for northern cod of reproductive age from 1972 to 1992 with variability generally increasing with age (Fig. 13). In general, weight-at-age increased from the early 1970s to the late 1970s before declining thereafter. The lowest weights-at-age occurred between 1972 and 1975 for all age classes except the 10- and 11-yr-olds whose lowest weights-at-age were experienced in 1992. Although these commercial data represent the longest continuous time series of weight-at-age data for northern cod, it should be noted that the representation of cod from each division differs somewhat annually.

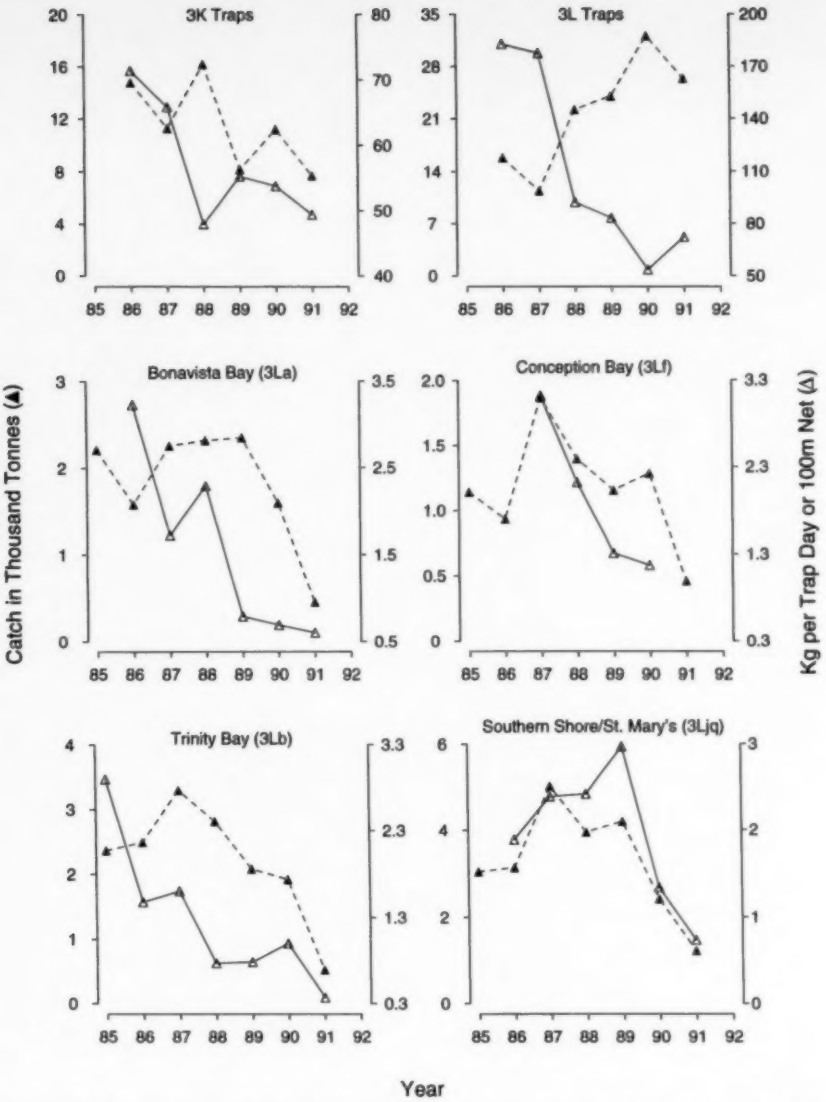

FIG. 11. Inshore catches (solid triangles) and catch rate (open triangles) for the trap (3K and 3L) and gillnet (3L unit areas) fisheries for northern cod.

Temporal changes in age structure are evident from age-specific variation in fecundity contribution to the northern cod stock (i.e., age-specific abundance multiplied by age-specific fecundity; Fig. 14). Independent of time, most eggs are produced by 6-, 7-, and 8-yr-old females. The fecundity contribution of older individuals has declined dramatically since the 1960s (1962–69) when an annual average 30% of all eggs (46% in 1962) were produced by 10- to 14-yr-old cod. This percentage dropped by almost one half to 17% in the 1970s before declining further to 12% in the 1980s and 11% in the last 2 yr of the fishery (1990 and 1991). Changes in age structure during the last 5 yr of the fishery (1987–91) were very similar to those of the 5 yr leading up to the extension of Canada's jurisdiction to 200 miles (1973–77; Fig. 14, lower two panels). In addition to similar collapses in age structure, total estimated fecundity declined 23% during both 5-yr periods.

Annual Variation in the Realized Rate of Natural Increase

Age-specific survival probabilities imposed on northern cod since 1962, and corresponding age-specific fecundities, have rarely been sufficiently high to allow northern cod to sustain themselves (Fig. 15). Independent of the survival estimate to age 3 (Fig. 15A and 15B), the annual rate of change in cod abundance declined steadily from 1962 to 1976, increased to 1980 following Canada's jurisdictional extension to 200 miles, and then declined again from 1981 to 1991. The two periods in which r rose dramatically, i.e., 1976–80 and 1991–92, were associated with significant reductions in fishing pressure by non-Canadian and Canadian vessels, respectively.

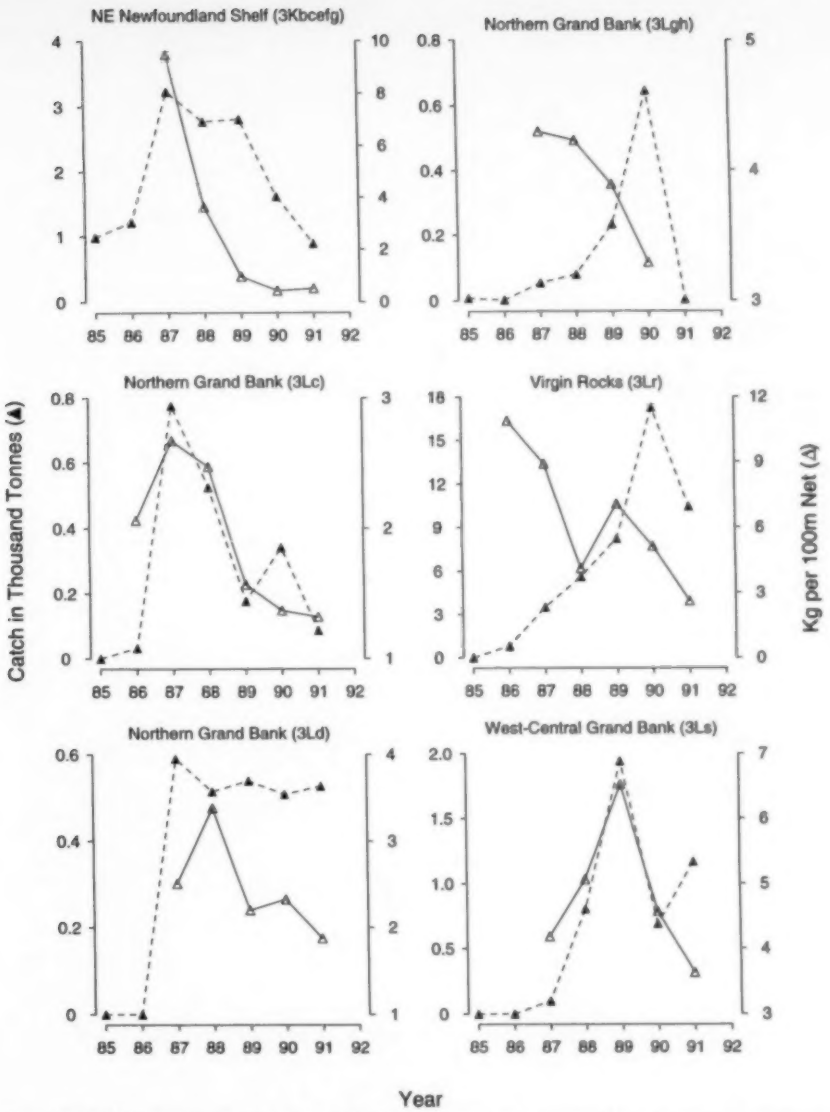

FIG. 12. Offshore gillnet catches (solid triangles) and catch rate (open triangles) in 3K (all offshore unit areas combined) and in six unit areas throughout 3L. See Fig. 1 for location of unit areas.

Harvestable abundance (cod aged 3 yr and older) from 1962 to 1992 estimated from yearly estimates of r, independent of survival to age 3 yr, is highly correlated with estimates based upon the VPA (Fig. 15C; 1962–77 data from Baird et al. 1991a; 1978–92 data from Bishop et al. 1993b). Although the VPA- and r-based estimates of abundance are not independent (both use the same survival data), their concordance suggests that predicted changes in population sustainability reflect temporal changes in cod abundance.

Discussion

Collapse of the Northern Cod Stock

The demographic changes that northern cod have incurred since 1962 have rarely allowed the stock to sustain itself over the past three decades. This lack of sustainability coupled with historically high commercial catches and technological expertise, an age structure that has collapsed with respect to number of reproductive age classes and numbers of individuals per age class, declining inshore and offshore catch rates during the past decade, and a lack of evidence for environmentally related influences on cod survival lend overwhelming support to the hypothesis that human overexploitation precipitated the commercial extinction of northern cod.

Unsustainable Harvesting of Northern Cod

The inability of northern cod to achieve sustainability is not surprising given the harvest rates (i.e., commercial catch/harvestable biomass) imposed on the stock since 1962

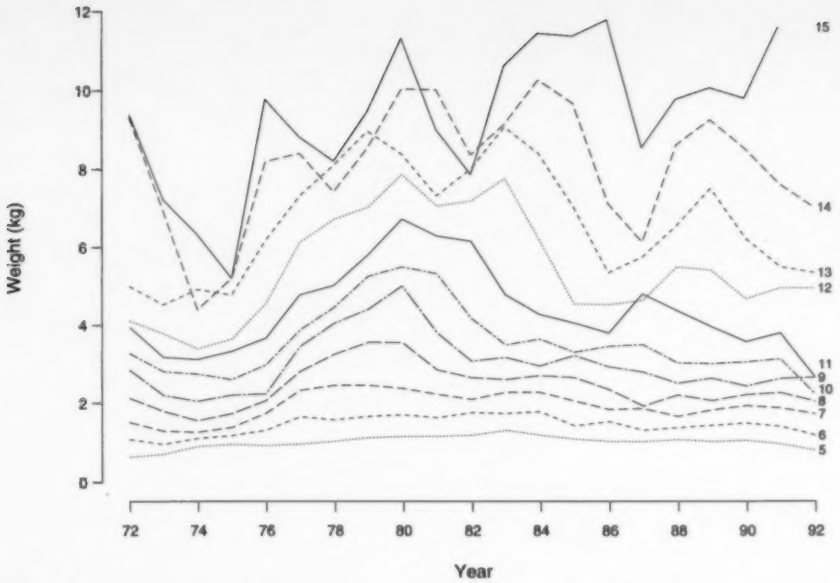

FIG. 13. Weight-at-age data for northern cod of ages 5–15 yr as determined from commercial catches from 1972 to 1992.

(Fig. 16A). A comparison of Fig. 15A, 15B, and 16A indicates that harvest rates less than 17% (the 1962 estimate) will permit the stock to sustain itself. The approximate linearity of the stock–recruit relationship for northern cod (Fig. 3A) suggests that the sustainable harvest rate will be relatively constant with changes in stock size. The data suggest that the stock was sustainable at harvest rates between 19 and 25% from 1978 to 1984. However, this interpretation should be treated with caution, as this period coincided with the largest age-specific weights, and hence the largest age-specific fecundities (and thus increased r), since 1972. These large weights-at-age may be attributable to the high growth rates associated with low density for northern cod (Millar and Myers 1990) and may not be representative of the average age-specific weights (or fecundities) experienced by the stock. It is also noteworthy that age-specific weights in the late 1980s and early 1990s are similar to or greater than those recorded between 1947 and 1950 (Fleming 1952). It should be noted that our estimates of harvest rate are probably underestimates because they do not include age-specific vulnerabilities to the fishery (younger fish tend to be less vulnerable to the fishery than older fish).

A total allowable catch (TAC) was first established for northern cod by the International Commission for the Northwest Atlantic Fisheries (ICNAF) in 1973 (temporal changes in management criteria for northern cod are detailed by Steele et al. (1992) and by Lear and Parsons (1993)). The TAC was based upon the management strategy of fishing at the maximum sustainable yield, i.e., at $F_{max} = 0.35$. Increased concern about the conservation of the resource led to the establishment of $F_{0.1}$ (described by Gulland and Boerema 1973) as the target fishing mortality rate in 1977. The $F_{0.1}$ for northern cod was set at 0.2 which corresponded to a harvest rate of 18%. TACs were set at the $F_{0.1}$ level in 1977 and 1978, at 80% $F_{0.1}$ from 1979 to 1983, and again at 100% $F_{0.1}$ from 1984 to 1986. Following 2 yr during which TACs were once again based upon fishing mortality less than $F_{0.1}$, it was realized that fishing mortalities throughout the 1980s had consistently exceeded $F_{0.1}$ because of overestimation of stock size (Baird et al. 1991b). Concomitant with these excessive fishing mortalities were the high proportions of the commercial catches composed of largely prereproductive individuals, i.e., less than 7 yr of age. These percentages increased from 55% in each of 1980, 1981, and 1982 to a mean of 66 ± 4(SE)% from 1983 to 1992 (range 46–84%) (data from annual stock assessment documents for the 2J3KL stock). The extent to which TACs since 1977 have exceeded the sustainable harvest rate of 17% suggested here is illustrated in Fig. 16B.

Perhaps the most direct question related to the relative importance of overfishing to the recent collapse of northern cod is: How much of the variation in abundance in the past decade can be explained solely on the basis of changes in harvest rate? Although a temporal analysis of changes in fishing mortality in the 1980s indicates that harvest rates were exceedingly high, these harvest rate data are based upon estimates of age-specific abundance derived from VPA in which natural mortality is assumed to be constant.

One means of assessing the validity of the harvest rates estimated for the 1980s is to calculate changes in the abundance of northern cod based upon changes in harvest rate and then compare these with independent estimates of abundance such as those derived from the autumn research surveys. Annual changes in abundance N from year t to year $t + 1$ as a consequence of changes in harvest rate in year t, H_t, can be estimated as

$$(5) \qquad N_{t+1} = N_t\, e^{r*}_t\, (1 - H_t).$$

Thus, abundance is predicted to increase between years t and $t + 1$ by the expected rate of population increase *in the absence of fishing* in year t, e^{r*}_t, and to decline by an amount proportional to the harvest rate experienced in that

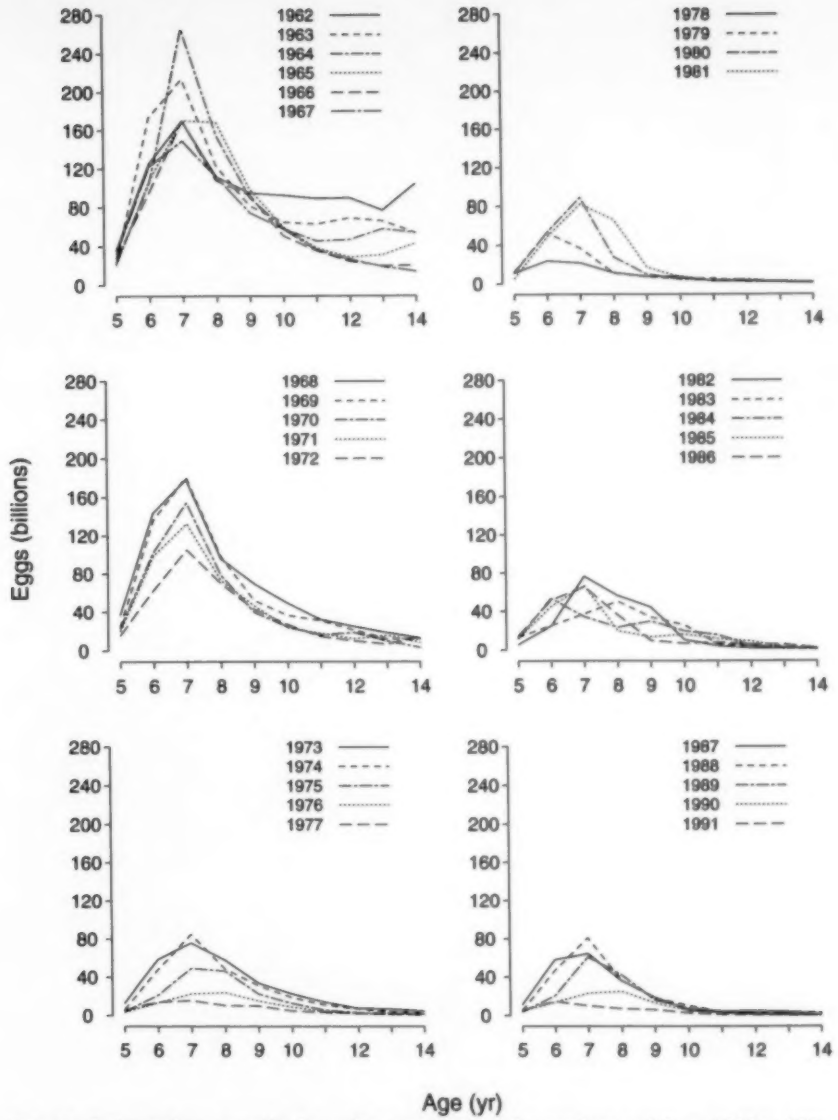

FIG. 14. Estimated age-specific fecundity contributions for northern cod from 1962 to 1991.

year (cf. Lockwood 1987). e_t^{r*} is estimated by assuming constant natural mortality of 18% (i.e., $M = 0.2$) and no fishing mortality (i.e., $F = 0$).

The longest continual decline in abundance of exploited northern cod in the 1980s occurred from 1985 to 1992 (Fig. 15C). Predicted changes in abundance during this period, as estimated from Equation (5), can be compared with those derived from the autumn research surveys (for which annual changes in abundance are a consequence of both natural and fishing mortality). To account for sampling error, observed mean number of harvestable cod per tow in year t was approximated by the mean number per tow in years $t - 1$, t, and $t + 1$, i.e., as a 3-yr running mean (data from Bishop et al. 1993b). The predicted numbers of cod per tow are highly correlated with those observed during the research surveys ($r = 0.78$, $p = 0.02$, p-value corrected for autocorrelation; Fig. 17). This concordance suggests that 61% of the variation in abundance from 1985 to 1991 can be accounted by harvest rate and lends strong support to the hypothesis that the decline of northern cod in the 1980s can be attributed primarily to overexploitation. This conclusion is supported by an independent analysis which indicates that the natural mortality of northern cod did not increase prior to the imposition of the moratorium in 1992 (Myers and Cadigan 1995).

Long-term Demographic Collapse of Northern Cod

By any demographic measure, the viability of northern cod has been seriously threatened since the introduction of European-based offshore trawlers in the late 1950s and early 1960s. By 1977, when compared with 1962 estimates, (1) harvestable biomass had declined 82% (2 961 000 versus

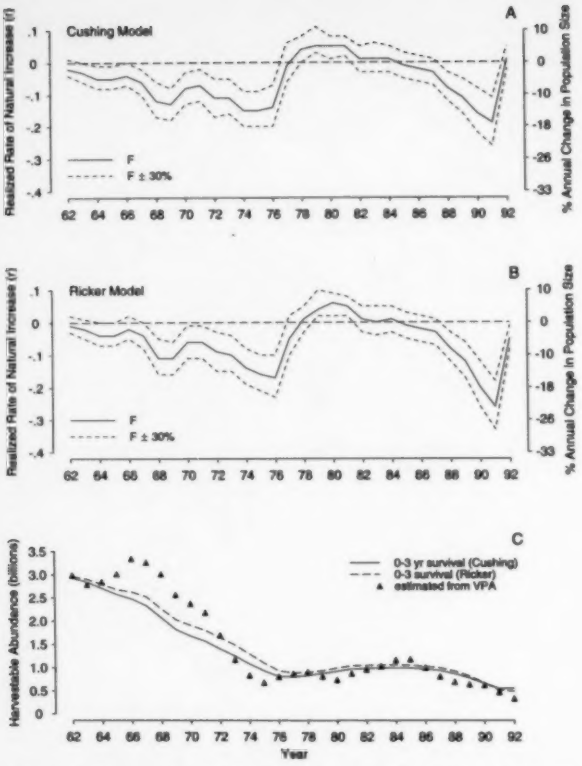

FIG. 15. Temporal variation in r, the realized rate of natural increase (left ordinate), from 1962 to 1992 and its relationship to harvestable abundance (cod aged 3 yr and older) from 1978 to 1992. At $r = 0$, the population replaces itself through time. When r is greater or less than 0, the population increases or decreases, respectively, at the annual rates of change indicated on the right ordinate (note that the right ordinates are not linearly scaled). (A) Survival from birth to age 3 yr estimated from the Cushing spawner–recruit relationship. (B) Survival from birth to age 3 yr estimated from the Ricker spawner–recruit relationship. (C) Temporal change in harvestable abundance as estimated by VPA (Bishop et al. 1993b) and as calculated assuming annual rates of change in population size corresponding to the annual estimates of r with survival from birth to age 3 yr estimated from the Ricker and Cushing stock–recruit relationships.

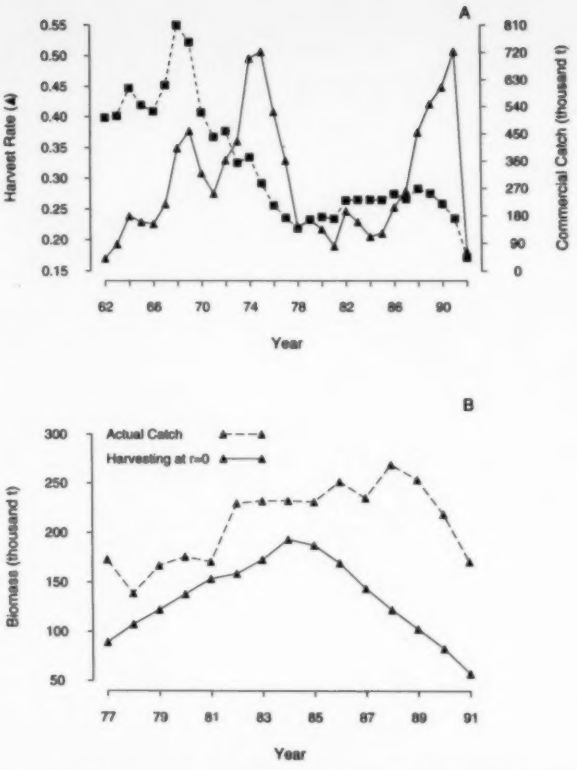

FIG. 16. (A) Temporal variation in harvest rate (commercial catch/harvestable biomass) of commercial vessels (triangles) and in reported commercial catches (squares) from 1962 to 1992. (B) Changes in actual and estimated sustainable catches (harvest rate = 17%, see text) for northern cod from 1977 to 1991.

526 000 t), (2) spawner biomass had declined 94% (1.6 million versus 93 000 t), (3) recruitment had declined 84% (1 billion versus 168 million), (4) fecundity contribution of 10- to 14-yr-old fish had declined from 46 to 18%, and (5) harvest rates had yet to attain levels at which the stock was sustainable. For most populations, the 82% reduction in harvestable northern cod biomass experienced between 1962 and 1977 would have been considered a collapse! If not for the large reduction in harvest rate by 1978 (less than half the 1975 rate of 51%) concomitant with the establishment of the 200-mile limit and the fortuitously high recruitment (relative to spawner abundance) provided by the 1978–81 year classes (cf. Fig. 7), northern cod would probably have collapsed by 1980. The remarkably similar patterns of decline in r (Fig. 15), in age-specific fecundity contributions, and in total fecundity between the 1973–77 and 1987–91 time periods (cf. Fig. 14, lowest two panels) are consistent with this hypothesis. Given the near collapse of the stock in 1977, it is not surprising that there was little margin for error in establishing sustainable harvest rates in the early 1980s. However, harvest rates in the 1980s greatly exceeded the targeted $F_{0.1}$ level, largely because of overestimation of stock size (described by Baird et al. (1991b) and by Hilborn and Walters (1992, p. 533–535) and discussed further below) coupled with great uncertainty in abundance estimates derived from the research surveys (standard errors of 30–40% of the mean are not uncommon; data in Bishop et al. 1993b).

Changes in Catch Rate and Spatial Allocation of Effort as Metrics of Stock Health

The decline in northern cod abundance in the 1980s was reflected by changes in fishing effort and catch rate. During the decade preceding the northern cod moratorium, a twofold decline in annual survival probability (cf. Fig. 2) was associated with increased inshore and offshore fishing effort, a spatial change in allocation of gillnet effort, and dramatic declines in catch rate of inshore and offshore fishing gear. The observed doubling of hours trawled and the halving of trawler catch rate may be conservative estimates of the temporal increase in fishing effort and decline in harvesting efficiency (cf. Rothschild 1977). One hour of trawling in

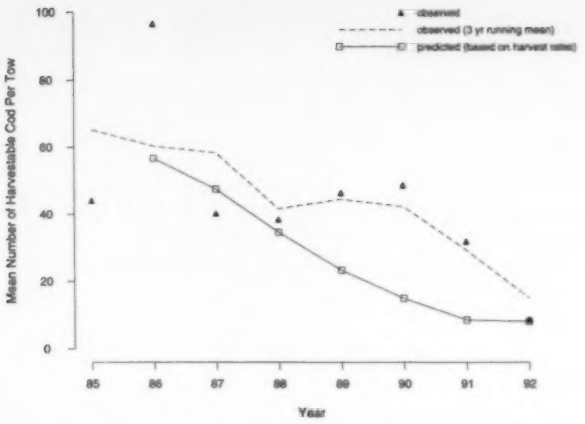

FIG. 17. Mean number of harvestable cod (cod aged 3 yr and older) per tow in 2J3KL from 1985 to 1991 as observed from autumn research surveys and as predicted from annual estimates of harvest rate.

1991 may have exerted considerably more mortality on the stock than one trawling hour in 1981 because of increased ability to locate and harvest cod through advances in technology and learning by those exploiting the resource. Temporal changes may also, however, have been affected by changes in trawling practices and gear operation which can combine to decrease trawling effort (e.g., the employment of windows on trawls to allow for increased escapement of fish).

Increasing inshore catches combined with declining catch rates were indicative of dramatic increases in effort in the mid- to late 1980s (and possibly as early as the 1960s; cf. Templeman 1966). Initiation of the use of gill nets offshore in 1987, where catch rates declined at even faster rates than they did inshore, would appear to have been a direct consequence of declining catch rates inshore. Thus, despite increases in both gillnet and trap catches of northern cod from the mid-1980s (Fig. 10; Bishop et al. 1993b), these patterns did not reflect the health of the stock because they obscured the underlying dynamics of spatial and temporal changes in the allocation of effort.

The influence of changes in gear technology on catch rates of northern cod, and on management's perception of stock health, has not received the attention it warrants. It is clear that the ability of inshore fishers to catch fish has increased dramatically with time, particularly during the 1980s, and any interpretation of temporal changes in landings should reflect these changes in effort. For example, echo sounders can permit the employment of greater number of cod traps per fisher because of the avoidance of hauling empty traps. Sounders also reduce the time handline fishers spend on locating cod. Throughout the 1980s, there was increased use of "Japanese" cod traps whose design allows for reduced escapement of fish and permits their placement in areas unsuitable for regular cod traps (Neis 1992). Advanced navigation equipment such as Loran C enables fishers to record electronically the location of large fish assemblages, sites of previously large catches, and the precise orientation of gillnet sets. The necessity of having to increase effort significantly throughout the 1970s and 1980s in order to maintain catches is amply reflected in interviews with inshore cod fishers (e.g., Neis 1992; Davis et al. 1994).

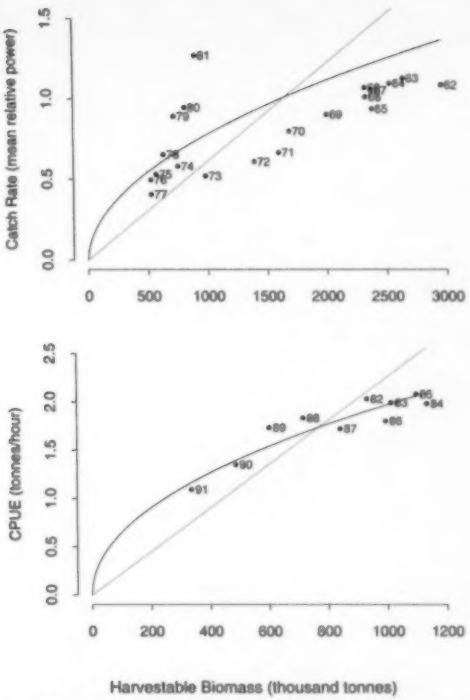

FIG. 18. Relationship between catch rate (estimated from a multiplicative model by Bishop and Gavaris 1982) and harvestable biomass of northern cod (biomass of cod aged 3 yr and older) from 1962 to 1981 (upper panel) and between nominal catch per unit effort (CPUE) of Canadian stern otter trawlers and harvestable biomass from 1982 to 1991 (lower panel).

High trawler catch rates can be maintained despite declining stock size for the simple reason that fishers do not exploit fish in a random manner. Increased ability to locate large assemblages of fish coupled with an apparent increase in the concentration of northern cod in the 1980s (Bishop et al. 1993b) can also lead to nonlinear relationships between catch rate and stock abundance, thus violating one of the implicit assumptions of most analyses of commercial catch data (Hilborn and Walters 1992). Such nonlinearity is evident in plots of catch rate against harvestable biomass of northern cod during the 10 yr immediately preceding the moratorium (1982–91) and during the previous 20 yr (1962–81). (Fig. 18). Catch rates from 1982 to 1991 represent the nominal CPUE estimated from 500–1000 GRT Canadian trawlers; catch rates from 1962 to 1981 were those estimated by Bishop and Gavaris (1982). For both time periods, a curvilinear relationship between CPUE and harvestable biomass (HB) ($CPUE = \alpha HB^{\beta}$) provided a significantly better fit to the data than did the model with β constrained to be 1 (1962–81 data: $CPUE = 0.001HB^{0.52}$, $F_{[2,17]} = 64.6$, $p < 0.00001$; 1982–91 data: $CPUE = 0.070HB^{0.48}$, $F_{[1,8]} = 69.3$, $p = 0.00003$) such that the probability of overestimating abundance increases as CPUE declines (note the similarity in the slopes, β, of the two models). The first-order autocorrelation coefficient was significant ($p = 0.0006$) in the 1962–81 data but not for the 1982–91 data. Thus, the parameters for the model describing the 1962–81 data were estimated under the assumption that the error term is an autoregressive process.

We also assumed that all estimation errors were in CPUE (following Richards and Schnute 1986). If estimation errors are assumed to be in HB, similar and highly significant regressions are obtained. The pattern of nonlinearity illustrated in Fig. 18 is expected in fisheries (such as the offshore trawler fishery for northern cod) where search is highly efficient, effort is concentrated in areas in which fish are most abundant, and fish remain concentrated as abundance declines (Hilborn and Walters 1992). There is a growing literature which indicates that β is less than 1 for many gadoid stocks with the result that fishing mortalities can remain high despite declines in stock size (e.g., Cooke and Beddington 1985; Crecco and Overholtz 1990; Rose and Leggett 1991).

Increased search and harvesting efficiency of Canadian trawlers contributed to the serious overestimates of northern cod abundance in the 1980s. This is well illustrated from 1977 to 1981 (Fig. 18, upper panel) in which a more than threefold increase in catch rate was associated with a relatively small increase in stock abundance. The potential for overestimation of stock size is clear given that technological advances had increased catch rates from 1979 to 1981 to levels previously realized in the 1960s when abundance was considerably higher. From 1978 to 1986, the abundance of northern cod was usually approximated by the midpoint of the VPA estimates derived from both commercial CPUE data and data from research vessel surveys (Baird et al. 1991b). However, abundance estimates based upon the commercial data indicated that the stock had increased threefold from 1978 to 1988 whereas those based upon the research surveys suggested that the stock had changed little in size (Baird et al. 1991b; Hilborn and Walters 1992, p. 533–535). The main consequence of this overestimation of stock size was that actual fishing mortality rates exceeded the targeted $F_{0.1}$ level of $F = 0.2$ more than twofold from 1978 to 1983 (average of $F = 0.41 \pm 0.03$ (SE) for cod aged 7–9 yr) and more than threefold from 1984 to 1989 (average of $F = 0.65 \pm 0.08$) (data in Bishop et al. 1993b).

Relative Importance of Environmental Change to the Collapse of Northern Cod

Water temperature and sustainable harvests

How much of the variation in northern cod abundance in the decade preceding the moratorium can be attributed to variability in the environment? More specifically, to what extent did "ecological factors" in 1991 (Lear and Parsons 1993) or cold temperatures throughout the 1980s (deYoung and Rose 1993) predicate the collapse in cod abundance? Long-term data for a variety of environmental indices including the NOA index (data on sea level air pressure) (Drinkwater 1994), areal extent of the CIL on the Northeast Newfoundland Shelf (Drinkwater 1994), Station 27 water temperatures (Fig. 4), 100 m depth water temperatures throughout 3L, pack ice persistence off Labrador, and air temperature in St. John's (Fig. 5) indicate that while it was relatively cold and ice cover relatively extensive in 1991, similar conditions had been experienced during the previous two decades and considerably colder conditions had prevailed throughout the nineteenth and early twentieth centuries. Evidence of a colder environment in the 1800s is also supported by ground borehole data from Newfoundland (Beltrami and Mareschal 1992) and by long-term (1550–1978) tree-ring data from northern Canada and Alaska (which indicate that most of the nineteenth century was characterized by slower than average growth whereas better than average growth has occurred since 1925; Jacoby and Cook 1983).

Despite the colder environment from 1800 to the 1920s, it was possible to sustain a fishery of the size that was not sustainable in the 1980s. There is no evidence of extraordinarily high natural mortalities leading to a major stock collapse in the past two centuries of the kind experienced in the early 1990s. Extant historical records of mass cod mortalities (Templeman 1965) were reported during years (1876, 1934, 1960) in which catches were relatively high (~160 000, ~260 000, and 459 000 t, respectively). If the reduction in cod abundance in the 1980s and early 1990s was due to increased natural mortality resulting from cold temperature, then similar population declines should have been evident during years with similarly cold temperatures (e.g., 1972–74, 1984–85, 1991–92). Despite encompassing all three cold periods, the longest continous research time series (in region 2J) did not yield a significant correlation between water temperature and cod abundance. It is also worth noting that the large 1.7°C decline in water temperature on St. Pierre Bank (NAFO division 3Ps) off the south coast of Newfoundland from the 1981–84 average of 1.4°C to the 1985–91 average of −0.7°C (mean temperature at 50 m between January and June; data in Hutchings and Myers 1994) was not associated with a precipitous decline in cod biomass (Bishop et al. 1993a).

Water temperature and depth distribution of cod

Data from the autumn research surveys are not consistent with a directed movement of large numbers of cod to deeper water (Fig. 8). Any apparent increase in the proportion of cod biomass in deep water in the late 1980s and early 1990s can be attributed to the near absence of cod in shallow water. In addition, the distribution of cod with depth appears to be unrelated to water temperature. Although the water temperature data in our analysis were those from Station 27, and as such do not extend to some of the deeper depths at which fish were caught in the research surveys, our results agree with those of Lilly (1994) who examined the relationship between bottom temperatures recorded at the time of the research surveys and the abundance of cod in the shallowest strata in division 2J. He concluded that there was no support for the postulate that the reduced abundance of cod in the north and west sections of 2J and 3K was the result of avoidance of cold water. Hutchings and Myers (1994) could also find no association between late and winter/spring temperatures and cod biomass on the shelf relative to that on the slope in 3L (the latter being a metric of the spring migration from the slope to shelf).

Water temperature and recruitment

We are unable to document any association between water temperature and recruitment of cod to the fishery (Fig. 7). Of the five cold years for which data are available (1972–74, 1984, 1985), two year classes were greater than average whereas three were less than average (Fig. 7, lower panel). Myers et al. (1992) were also unable to find any consistent relationship between Station 27 water temperature or the extent of the CIL with either VPA or research survey estimates of recruitment. In contrast, deYoung and Rose (1993) reported a positive correlation between northern cod recruitment and temperature between 1972 and 1988. Their exclusion of data from 1962 to 1971 notwithstanding, deYoung and Rose (1993)

removed the effects of spawner abundance on recruitment by dividing the number of 3-yr-olds by the *number* of cod aged 6 yr and older. This means of accounting for changes in the fecundity of a stock can be justified only if the age and size distributions of reproductive individuals remain constant with time, i.e., if fecundity is a function of the numbers of individuals and neither their age nor size. It is clear, however, that both mean age and body size have declined over the past two and three decades (cf. Fig. 14). Decreasing age and body size will have the effect of continually underestimating recruitment with ever-increasing bias when number of recruits is divided by number of spawners. If the effect of spawner *biomass* (a more reliable metric of fecundity than abundance) on recruitment is removed from the analysis, there is no significant association between recruitment and water temperature (depth-averaged over 0–50 m) for the 1972–89 year classes of northern cod ($r = 0.31$, $p = 0.210$). Again, if water temperature in the upper 50 m (the usual location of cod larvae; Anderson and deYoung 1993) has a significantly detrimental effect on larval survival, a significant decline in recruitment in 3Ps should have been observed following the 1.4°C decline in temperature (greater than *any* annual decline recorded at Station 27) at 50 m between 1984 and 1985. However, there is no significant association between water temperature (January through June at 50 m; data in Hutchings and Myers 1994) and the residuals from the regression of $\log_{10}$(recruitment) against $\log_{10}$(spawner biomass) (data in Bishop et al. 1993a) for cod in 3Ps from 1972 to 1989 ($r = 0.11$, $p = 0.662$).

It has been argued that cold temperatures effect southerly shifts in the location of spawning cod and lead to recruitment failures (deYoung and Rose 1993). Empirical support for the argument is primarily of three kinds. The first was an apparent association between water temperature and recruitment which, as outlined above, is problematic in its interpretation. Second, cod larvae in 2J3KL were purported to be more southerly distributed in a cold year (1935) than in a warm year (1934). However, this observation must be tempered by the observation that there was no difference in water temperatures on the Northeast Newfoundland Shelf in 1934 and 1935 (MEDS data base; $t_{[76]} = 0.95$, $p = 0.35$). Third, deYoung and Rose (1993) also argued, citing data on spring distributions of cod on the Northeast Newfoundland Shelf, that cod were more southerly distributed in two cold years (1984 and 1985) than in two warm years (1986 and 1987). However, given that the hydroacoustic samples upon which these data are based were not conducted north of 51°30′N in 1984 and 1985 because of ice conditions, the finding of cod north of this latitude in 1986 and 1987 does not constitute evidence that cod are more northerly distributed in warm years (a caveat noted also by deYoung and Rose 1993). The claim that cod are more widely dispersed in warm years cannot be assessed in the absence of data on actual locations and sampling effort of the ship(s) involved.

Salinity and recruitment

Even if there is an effect of water temperature on recruitment, although not detectable with data presently available, it will likely be small relative to that of the size of the spawning stock. This can be illustrated by an analysis of the effects of salinity on northern cod recruitment. Based upon a model proposed by Sutcliffe et al. (1983), Myers et al. (1993) found that salinity experienced during the second year of life was significantly related to recruitment from 1976 to 1987 when spawning stock biomass was included in the analysis. We repeated their multiple regression analysis because the most recent VPA estimates of recruitment and spawner biomass (Bishop et al. 1993b) indicated that recruitment from the 1984 to 1988 year classes was substantially less than had been estimated in 1992 (Baird et al. 1992). The percentage reductions in the 1993 recruitment estimates relative to those from 1992 were 16% (1984 year class), 23% (1985), 42% (1986), 55% (1987), and 43% (1988).

Our reanalysis indicates that salinity has little effect on recruitment relative to that of spawner biomass. Comparing one-variable models, the r^2 of the regression of log-transformed spawner biomass against log-transformed recruitment ($r^2 = 0.39$, $p = 0.0004$; untransformed recruitment and spawner biomass data: $r^2 = 0.73$, $p < 0.0001$) is highly significant whereas that of the regression of salinity against log-transformed recruitment is not ($r^2 = 0.11$, $p = 0.08$; untransformed recruitment data: $r^2 = 0.15$, $p = 0.04$). The marginal significance of salinity suggested by the regression of salinity against the untransformed recruitment data is not evident in the multiple regression model (i.e., recruitment = α(spawner biomass) + β(salinity) + ϵ). Following the stepwise addition of spawner biomass, inclusion of salinity does not significantly improve the r^2 of the multiple regression for either the transformed ($p = 0.10$) or untransformed data ($p = 0.07$).

Resistance and resilience to environmental variation

We conclude that, relative to the effect of spawner biomass, neither temperature nor salinity had a significant influence on recruitment of northern cod prior to the moratorium in 1992. However, one consequence of the dramatic reductions in abundance experienced by northern cod over the past three decades may have been increased susceptibility to environmentally induced stochasticity in age-specific survival rates (Harrison 1979; Lande 1988). Populations with low r can have reduced ability to return to their equilibrium density following a disturbance from equilibrium, i.e., they have low resilience (Holling 1973; May et al. 1974; Harrison 1979; Pimm 1991). Low resilience of northern cod is implied by their low intrinsic rates of increase during the 1970s and mid- to late 1980s (Harwood 1978; Harrison 1979). Thus, the negative association between resilience and harvesting mortality (Beddington 1978; Harwood 1978) may have increased the susceptibility of cod in recent years to changes in water temperature to which larger, more resilient populations would not have been affected. Thus, the extremely low abundance of northern cod in the 1990s raises justified concern that increased vulnerability to both fishing (within and without the 200-mile limit) and naturally occurring environmental variation may significantly increase the probability of extinction of this stock.

Population resilience would also be expected to decline with increased variability in juvenile survival. Based upon their finding that age is positively correlated with spawning duration in cod, Hutchings and Myers (1993) identified a mechanism by which the size-selective mortality against larger, older individuals characteristic of most fisheries can increase variability in recruitment. Selective mortality against larger individuals is reflected by the observation that relative to the early 1960s, the expected fecundity contribution of older cod (15–20 yr) in the late 1980s had declined by two orders of magnitude relative to that of younger (7–9 yr)

individuals, i.e., for every 100 eggs produced by younger cod, older cod produced 40 eggs in the early 1960s but only 4 eggs in the late 1980s (Fig. 1B in Hutchings and Myers 1993). Given that younger cod spawn for a shorter period than older cod (Hutchings and Myers 1993), this bias towards younger ages coupled with a decline in the number of age classes during spawning would be expected to shorten the effective spawning season, reducing the likelihood that larvae could initiate feeding during the period of peak zooplankton abundance and thereby increasing variation in juvenile survival.

Conclusions

Our analyses of realized population growth (r) indicate that northern cod have rarely been fished at sustainable levels since at least 1962. Overfishing by long-distance trawlers in the 1960s and 1970s had reduced the stock to near commercial extinction in 1977. Since Canada's extension of fisheries jurisdiction to 200 miles, two factors were of prime importance in contributing to the overexploitation of the stock from 1977 to 1991. First, management estimated that recruitment from 1977 would equal the average maintained from 1962 to 1972 (see Steele et al. (1992) and Lear and Parsons (1993) for more details). Given that the stock (i.e., harvestable abundance) was three to four times larger in the 1960s than in 1977, and that recruitment was *not* corrected for changes in spawner biomass, the predicted rate of growth of the stock was greatly overestimated (see harvest predictions in Kirby 1982). Nonetheless, the predicted growth of the stock led to markedly increased industrial and government investment which fuelled a socioeconomic and political optimism in the fishery whose momentum could not easily be abated. The second factor was the overestimation of stock size (described earlier) and the concomitant excess of actual fishing mortality over targeted rates. As noted by Finlayson (1994), reliance upon commercial data, whose catch rates indicated that the stock was increasing in size, to "fine-tune" the VPAs (at least until 1986) was probably a reflection of management's prediction of rapid growth of the stock in the late 1970s.

The temporal changes in demography, population sustainability, harvest rates, and inshore/offshore catch rates documented here provide strong evidence that overexploitation was the primary cause of the collapse of northern cod in the early 1990s. The inability to identify unambiguous and significant associations between water temperature and both spatial and depth-related indices of juvenile and adult cod abundance (Myers et al. 1992; deYoung and Rose 1993; Lilly 1994; present paper), the collapse in population structure defined primarily by fewer reproductive age classes and fewer individuals per age class (Fig. 14), the observation that cod did not have anomalously low age-specific weights in the early 1990s (Fig. 13; Fleming 1952), the observation that the population decline can be predicted solely from harvest rates and cannot be attributed to increased natural mortality (Fig. 17; Myers and Cadigan 1995), the low amount of recruitment variability that can be attributed to water temperature and salinity, and the fact that large harvests were sustainable during a colder environment in the nineteenth and early twentieth centuries underscore our postulate that the environment contributed relatively little to the collapse of northern cod relative to that imposed by the fishery.

The cod collapse and previous commercial extinctions in the history of fisheries management (e.g., California sardine (Radovitch 1981); Peruvian anchovy (Hilborn and Walters 1992); African/Namibian pilchard (Cram 1981)) should provide ample justification for politicians, policy-makers, industry, and management to limit the urge to attribute resource collapses to vaguely understood or even imagined environmental causes. The ecological and socioeconomic consequences of so responding to repeated failure are too great.

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LETTERS AND COMMENTS/LETTRES ET COMMENTAIRES

Did Top-Down Effects Amplify Anthropogenic Nutrient Perturbations in Lake Michigan? Comment on Evans (1992)

An analysis of historic data by Evans (1992) questioned top-down control of either zooplankton or phytoplankton by alewife (*Alosa pseudoharengus*). According to the standard scenario, elimination of native lake trout (*Salvelinus namaycush*) stocks allowed successful invasion and initial population explosion of alewife, an exotic planktivore. Predation by alewife then depressed zooplankton populations, particularly large-bodied forms, that in turn allowed greater standing stocks of phytoplankton. Evans' findings of limited evidence for top-down control may be unconventional, but we do not find them surprising. In our view, changes in fish stocks were only one of the major changes in ecosystem dynamics that occurred coincidentally with and preceding the alewife population explosion and crash in the 1960s. Specifically, our research has shown that increased diatom production and biogeochemical silica depletion induced by anthropogenic phosphorus loading in the 1950s and 1960s resulted in a shift in phytoplankton community structure (Schelske and Stoermer 1971). Here we suggest several mechanisms by which the population explosion of alewife indirectly increased diatom production and accelerated biogeochemical silica depletion.

The explosive increase and subsequent crash of the alewife population occurred during a period (1955–70) when anthropogenic phosphorus loading to Lake Michigan increased dramatically (Chapra 1977) and when dissolved silica in offshore waters decreased approximately 3.0 $mg \cdot L^{-1}$ (Fig. 1). Several independent lines of evidence support the view that silica depletion resulted from increased diatom production stimulated by increased phosphorus availability in this phosphorus-limited system. These include experimental studies (Schelske and Stoermer 1971; Schelske et al. 1986), mass balance calculations (Schelske 1985), historic trends in major nutrients (Schelske 1988), and paleolimnology (Stoermer et al. 1990). The period of rapid silica depletion is coincident with increased sedimentation of diatom species that occupy the summer metalimnion, primarily *Cyclotella* spp. (Stoermer et al. 1990). Increase and subsequent decline of an oligotrophic *Cyclotella* association are often indicative of eutrophication in temperate lakes (Engstrom et al. 1985). The demise of this *Cyclotella* association signals depletion of epilimnetic silica in the Great Lakes (Stoermer 1993) and metalimnetic silica depletion in Lake Michigan (Schelske et al. 1986). All of these studies indicate rapid increases in diatom production during the 1950s and early 1960s and reduced diatom production, due to silica limitation, by the late 1960s. Thus the exponential population increase of alewife coincides with a period of increased diatom production, and the alewife crash in 1967 coincides with the beginning of a period of silica-limited diatom production.

Alewife were first reported in Lake Michigan in 1949 (Smith 1970). The potential impact of this species introduction on ecosystem dynamics is evident from the commercial catch that exceeded 1 million pounds by 1958, increased to 29 million pounds in 1966, and peaked at 42 million pounds in 1967 (Smith 1970). Furthermore, experimental trawling indicated a 56-fold increase in young-of-the-year from 1962 to 1964 and a fivefold increase in adults from 1962 to 1966 (Smith 1968). The population explosion of alewife undoubtedly depended either on food resources not used fully prior to invasion and establishment or to increased food resources. Exponential population growth of the magnitude reported for alewife is not sustainable under any condition and the population crashed in 1967.

Potential impacts of alewife on native fish stocks are heightened because adult alewife occupy different regions of the lake during different seasons, providing this species with a competitive advantage over other planktivores with more restricted habitats (Smith 1968). Young alewife (ages 1 and 2), however, occupy middepths throughout the year (Smith 1968), including the deep chlorophyll layer (Fahnenstiel and Scavia 1987) and the summer metalimnion where seasonal silica depletion occurs (see Schelske et al. 1986; Schelske 1988). One expected consequence of eutrophication during the period of rapid silica depletion is an increase in subsurface phytoplankton production below the mixed layer (Moll and Stoermer 1982). Our hypothesis is that metabolic activities of an expanding population of young alewife enhanced nutrient (phosphorus) turnover and diatom production in the metalimnion and in the photic zone below the metalimnion during the period of rapid silica depletion.[1] This source of recycled phosphorus for diatom production addresses the unresolved question of why silica depletion was so rapid at the time of the alewife explosion and why the response to increased nutrient loading did not occur sooner (see Schelske 1988). Thus, the population explosion of this exotic invader may have accelerated biogeochemical silica depletion already underway in the Lake Michigan ecosystem, but was not its primary cause.

The crash of the alewife population in 1967 coincided with the beginning of the present period of epilimnetic silica depletion (Fig. 1). This decrease in silica supplies (Schelske et al. 1986) resulted in reduced diatom production and altered the structure and composition of the phytoplankton community (Schelske and Stoermer 1971; Stoermer 1993). According to mass balance calculations, diatom production was six times larger during the period of rapid silica depletion than in the following period of silica limitation (Schelske 1985). Inferences from paleolimnological data indicate that subsurface production of diatoms decreased in the 1970s when diatom production was silica limited (Stoermer et al. 1990). Subsurface phytoplankton production is still important quantitatively in Lake Michigan but the importance of

[1]Note added in proof: The substantial role and importance of alewife in phosphorus regeneration has been estimated using a bioenergetics model of fish growth (C.E. Kraft. 1993. Phosphorus regeneration by Lake Michigan alewives in the mid-1970s. Trans. Am. Fish. Soc. 122: 749-755).

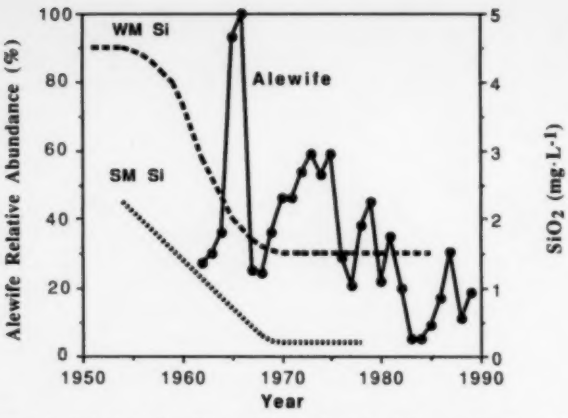

FIG. 1. Relative abundance of alewife and winter maximum and summer minimum concentration of silica in Lake Michigan from 1950 to 1989. After 1970, epilimnetic silica was reduced to levels (<0.2 mg $SiO_2 \cdot L^{-1}$) that limit diatom production. Data on alewife abundance after Evans (1992) and historic trends in silica after Schelske (1988).

diatoms decreases markedly soon after stratification (Fahnenstiel and Scavia 1987). Coincidence between reduced diatom production and alewife population dynamics since the crash in 1967, although interesting and possibly important, does not warrant speculation about causality.

We fully agree with Evans (1992) that evidence available is completely inadequate to weigh the possible importance of food chain effects objectively. A major part of Evans' analysis was possible only because a few unprecedented periods of large-scale measurement provided data on zooplankton abundance that could then be tested for correlation with alewife and phytoplankton abundance. Unfortunately, this type of data is only available for limited periods and areas of the lake. The most extensive data on phytoplankton abundance, for example, were collected at water filtration plants for Chicago and Milwaukee. These plants draw water from the nearshore region (about 5% of the lake volume) that differs chemically and biologically from offshore waters (see Schelske 1988). Consequently, sampling does not represent phytoplankton biomass in most of the water mass. Finally, adequately sampling planktonic or nektonic components of the water mass is compounded by the large area (58 000 km^2) and volume ($4.8 \times 10^{12} m^3$). These factors cause us to question whether limited historic data bases actually document changes in phytoplankton abundance and whether nearshore data collected at a few points represent the entire lake or only some restricted area of a very large system.

The relative importance of top-down versus bottom-up effects on historic ecosystem changes in Lake Michigan may never be quantitatively resolved. Classic examples of top-down control are obvious because they are supported by large changes in abundance. For example, small-bodied forms of zooplankton replaced larger forms after the invasion of alewife and then larger zooplankton again became abundant after the population crash of alewife in 1967 (Wells 1970). Our evidence for biotic change is based on diatom microfossils (Stoermer et al. 1990) and on annual rates of silica utilization for diatom production integrated over 15 yr (see Schelske et al. 1986; Schelske 1988). It may be more difficult, however, to measure biotic change during periods of greater system stability. Lehman (1988) concluded that predation by the recently introduced predaceous zooplankter *Bythotrephes cederstroemii* Schoedler had no measurable top-down effect on phytoplankton in the 1980s. This introduction, however, occurred during a period of reduced phosphorus loading. Other studies also indicate that the effect of zooplankton grazing on phytoplankton abundance in Lake Michigan (Evans 1992) or in other lakes (Turner and Mittlebach 1992) is not great. In addition, measurable impacts of top-down control may diminish progressively from the top of the food chain to the level of nutrient effects (see McQueen et al. 1989).

Our scenario on biotic change is that production of diatoms increased while the alewife population exploded, the opposite relationship advanced for top-down control. Thus, one important physiological component of the phytoplankton community increased instead of the expected decrease predicted by top-down control. Our analysis is based on diatom production and not on the usual abundance estimates. Although we have no reliable abundance estimates, we realize that abundance could decrease while production increases. Our scenario is unusual in that it is based on primary production by diatoms during a period of large increase in production of alewife.

The chemical environment and biological communities in Lake Michigan changed rapidly in less than one century. Dramatic changes already discussed and others not discussed here occurred on time scales of decades. Epilimnetic and metalimnetic silica reserves, for example, decreased rapidly to limiting levels for diatom growth in a 15-yr period and the alewife became established in less than a decade. We hypothesize that the expanding population of this exotic planktivore increased nutrient cycling that then accelerated growth and production of diatoms, and therefore suggest that biotic interactions played an important role in accelerating bottom-up effects that induced rapid silica depletion. We agree with Evans (1992) that other factors including spatial and interannual variability confound the analysis of causal factors responsible for historical biotic changes. Finally, we conclude that resolving factors controlling ecosystem change is more complex in large, spatially structured systems than in small, adequately sampled systems and that considering interactions among a complex of environmental factors is a necessary prerequisite instead of simply invoking top-down or bottom-up effects — Claire L. Schelske, *Department of Fisheries and Aquatic Sciences, University of Florida, 7922 NW 71st Street, Gainesville, FL 32653, USA*, and Eugene F. Stoermer, *Center for Great Lakes and Aquatic Sciences, University of Michigan, 2200 Bonisteel Boulevard, Ann Arbor, MI 48109, USA.* (J11979)

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Reply to "Did Top-Down Effects Amplify Anthropogenic Nutrient Perturbations in Lake Michigan?" by C.L. Schelske and E.F. Stoermer

Schelske and Stoermer (1994) hypothesize that the early to mid-1960s alewife (*Alosa pseudoharengus*) population explosion indirectly increased diatom production and thus accelerated biogeochemical silica depletion in Lake Michigan. This is thought provoking because it presents yet another hypothesis to the continuing discussions of how changes in alewife populations may have affected zooplankton and phytoplankton populations, water clarity, and now biogenic silicon. These discussions had their beginning with Wells' (1970) study which suggested that increased alewife abundances in summer 1966 had marked effects on zooplankton community structure. Wells also suggested that the spring 1967 alewife population crash allowed 1968 zooplankton community structure to regain features similar to that observed in summer 1955. Based largely on Wells' (1970) study of offshore zooplankton populations and long-term phytoplankton studies at the Chicago intake (Damann 1960; Makarewicz and Baybutt 1981), Edgington (1984, 1986) and Brooks et al. (1984) hypothesized that the increased alewife abundances during the late 1950s and the 1960s was the primary factor affecting increased phytoplankton abundances during this same period. This argument was not widely accepted and phosphorus abatement programs initiated in the early 1970s continued to be strengthened. Phosphorus levels declined as did phytoplankton abundances (Danforth and Ginsburg 1980; Makarewicz and Baybutt 1981), suggesting that phytoplankton abundances were strongly linked to phosphorus levels.

Support for the Edgington (1984, 1986) and Brooks et al. (1984) top-down argument began to increase during the early 1980s when alewife populations declined and large-bodied *Daphnia* increased in abundance in the offshore (Evans and Jude 1986). Summer water clarity was higher and chlorophyll concentrations were lower than in several preceding years, leading Scavia et al. (1986) to hypothesize that top-down control had enhanced the effects of phosphorus abatement programs in controlling algal biomass in Lake Michigan. However, later studies (Lehman 1988; Evans 1992) questioned this hypothesis. Evans (1992) also questioned the purported changes in zooplankton community structure as presented by Wells (1970) and the top-down argument for control of algal biomass during the 1960s as presented by Edgington (1984, 1986) and Brooks et al. (1984). Schelske and Stoermer's comment revitalizes the top down/bottom-up controversy for the late 1950s to late 1960s by asking researchers to consider the potential role of alewife in affecting silicon depletion rates in the offshore waters of Lake Michigan.

My first interest in reading Schelske and Stoermer's comment was to determine whether or not they, in developing their top-down hypothesis, now support Edgington's (1984, 1986) and Brook et al.'s (1984) top-down hypothesis. They apparently do not support the latter hypothesis. Rather, Schelske and Stoermer hypothesize that while diatom *production* was enhanced during a period of large increases in alewife abundance, diatom abundances could have decreased. This is a fascinating hypothesis and undoubtedly will stimulate new research into the role of top-down control mechanisms in affecting silicon depletion rates in Lake Michigan and other lakes. A variety of approaches can be used to test this hypothesis as outlined below.

First, the theoretical foundation for Schelske and Stoermer's silicon depletion hypothesis must be established. This foundation can best be established through experimental mesocosm or lake-manipulation studies. Experiments should be designed to investigate how the strength of silicon top-down control varies with trophic status and fish community structure. In investigating planktivorous fish, researchers should consider both exotics such as the alewife and indigenous planktivores such as the lake herring (*Coregonus artedii*) and bloater (*C. hoyi*).

The more difficult question to address is the degree to which top-down control affected silicon depletion rates in Lake Michigan from the late 1950s to late 1960s. Even with a strong theoretical foundation, Schelske and Stoermer's hypothesis will continue to be difficult to test because of the paucity of high-quality, quantitative data on the spatial and temporal patterns in fish, phytoplankton, and zooplankton abundances and in nutrient concentrations during this period. Despite these limitations, scientists continue to seek a better understanding of the driving factors for the rapid changes which occurred during this dynamic period. In further considering the causal factors for the silicon decline during the 1950s and 1960s, Schelske, Stoermer, and other researchers should consider the following important points.

(1) The scientific rationale for rejecting Edgington's (1984, 1985) and Brooks et al.'s (1984) top-down hypothesis while accepting Schelske and Stoermer's hypothesis requires further elucidation. If diatom biomass did not increase with increased diatom production, then diatom loss rates must have increased. Mechanisms must be proposed for this increased loss. For example, diatom loss rates could have increased with an increase in zooplankton grazing pressure or sedimentation rates. The former mechanism is unlikely because current research has shown that, as planktivores increase in

abundance, large-bodied zooplankton decline in abundance: zooplankton grazing rates decline and algal biomass increases. Thus, Edgington (1984, 1986) and Brooks et al. (1984) argued that increased alewife abundances during the 1960s affected a decrease in zooplankton grazing rates. Alternatively, diatom sedimentation rates could have increased during the late 1950s and early 1960s if diatom community structure shifted towards larger, heavier-celled species with more rapid settling rates. However, this shift in community structure probably would have been more strongly associated with seasons of strong vertical mixing of the water column (i.e., the spring and autumn diatom bloom) than the more quiescent conditions associated with the deep chlorophyll layer. Moreover, as silica concentration declined, diatom community structure should have shifted towards species with lower silicon content. Paleolimnological studies which examine shifts in diatom species composition, size, silicon content, and fragmentation (Glover 1982) should provide insights into the potential factors affecting increased loss rates of diatoms from the summer deep chlorophyll layer.

(2) More consideration needs to be given to the relationship between the rate of silicon depletion and increased alewife abundances over the late 1950s to mid-1960s. As presently described (Schelske and Stoermer, fig. 1), there is an insufficient linkage between the two variables. Although winter maximum silicon concentrations began to decline in the mid-1950s, the rate of decline decreased during the early to mid-1960s: meanwhile, alewife abundances continued to increase sharply. Summer minimum silicon concentrations decreased at an almost linear rate from the mid-1950s to late 1960s. If alewife had had a significant effect on the rate of silicon decline during the mid-1960s, the slope of the summer minimum and winter maximum silicon concentration curves should have become more steep during this period. Regrettably, silicon data are lacking for the mid-1960s (Schelske 1985, 1988) and so it will be difficult to further refine long-term trends in summer minimum and winter maximum silicon concentrations as shown in Schelske and Stoermer.

(3) More consideration needs to be given to the link between alewife abundances and silicon regeneration rates. Silicon is recycled relatively rapidly in Lake Michigan with only about 5% of biogenic silicon produced by diatoms buried in the sediments each year (Parker et al. 1977a; Schelske 1985). Thus, if increased alewife abundances enhanced silicon depletion rates from the late 1950s to mid-1960s, decreased alewife abundances in later years should have resulted in increased water column silicon concentrations. This apparently was not observed and requires explanation.

(4) Some consideration must be given to how annual silicon depletion rates are partitioned seasonally, i.e., how important are processes occurring in the summer deep chlorophyll layer relative to processes occurring in spring and autumn? Previous research has shown that most of the silicon depletion occurs during spring and early summer and is associated with the sedimentation of diatom frustules (especially large-celled taxa such as *Melosira islandica*, *M. granulata*, *Tabellaria flocculosa*, and *Stephanodiscus* spp. (Conway et al. 1977; Parker et al. 1977b; M.S. Evans, unpublished data). Because diatoms may account for a smaller proportion and absolute amount of summer versus spring algal biomass (Brooks and Torke 1977; Parker et al. 1977b), their summer contribution to annual silicon depletion rates may be small. Autumn may also be a season of significant diatom biomass and dominance (Brooks and Torke 1977; Parker et al. 1977b).

The relative contribution of spring versus summer diatom populations to silicon depletion could be investigated through a reexamination of the paleolimnological record for Lake Michigan. Stoermer et al. (1990) have shown that *Melosira* spp. and *Stephanodiscus* are the dominant diatoms preserved in the 1954–64 and later records. By estimating the biomass and silicon content of these and other preserved diatoms, a silicon budget could be developed to estimate species-specific contributions to annual silicon sedimentation and depletion rates. Moreover, by knowing the seasonal abundance patterns of these diatoms, the relative importance of seasonal events in annual (and long-term) silicon depletion rates could be assessed.

(5) Consideration also must be given to how interannual variability in climate may have affected silicon depletion rates. Researchers have hypothesized that interannual variability in weather has affected Lake Michigan water clarity, total phosphorus concentrations, chlorophyll concentrations, zooplankton composition, and long-term trends in alewife abundances (Eck and Brown 1985; Scavia et al. 1986; Eck and Wells 1987; Evans 1992). Silicon depletion may have been enhanced by events which favored prolonged diatom blooms (e.g., an extended spring with pronounced vertical mixing) or rapid sedimentation rates (e.g., a period of calm lake conditions following the establishment of the diatom bloom).

(6) Consideration also must be given to how increasing total phosphorus concentrations affected phytoplankton biomass and composition (edible versus inedible) and zooplankton biomass and grazing rates. As total phosphorus concentrations in Lake Michigan increased through the 1950s and 1960s (Chapra 1977), phytoplankton and zooplankton biomass also would have increased (Hanson and Peters 1984; Watson and McCauley 1988). According to Watson and McCauley's (1988) models, most of the increase in phytoplankton biomass would have been associated with increased inedible algae biomass. As zooplankton biomass increases, grazing pressure on edible algae intensifies, limiting edible algae biomass increases. Intensified grazing on small, edible diatoms may have enhanced silicon depletion rates, i.e., increased zooplankton biomass and grazing rates may have enhanced silicon depletion rates during the 1950s and 1960s independent of changes in alewife abundance.

(7) Researchers, in considering the role of increasing alewife abundances on the Lake Michigan ecosystem, must give consideration to the fact that indigenous planktivores occupied the lake prior to the alewife invasion. Important indigenous planktivores included the lake herring, bloaters, and other ciscoes. Overfishing, the sea lamprey (*Petromyzon marinus*) invasion, habitat alterations, etc., resulted in the decimation of these and other species (Christie 1974; Hartman 1988) and allowed for the alewife population explosion into niches vacated by indigenous planktivores. Researchers must consider whether or not alewife had a markedly different effect on the offshore lake ecosystem than indigenous planktivores.

(8) Greater consideration should be given to the influence of events occurring in the inshore in affecting the offshore record. The inshore region accounts for approximately 14% of the Lake Michigan surface area (Schelske 1977), and annual primary production rates may be approximately

1.7 times greater in the inshore than offshore (Vollenweider et al. 1974). Because the inshore may contribute as much as 21% to the annual primary productivity of Lake Michigan, events occurring in the inshore may have important consequences to the offshore.

Events occurring in the inshore during late winter and early spring may be particularly important in affecting the offshore. These periods correspond to the seasons when inshore-region algae (including diatoms) are most abundant (Damann 1966; Ladewski and Stoermer 1973; Danforth and Ginsburg 1980). Silicon is significantly depleted in the inshore region during this period (Schelske 1988). Silicon-depleted water is moved offshore with physical events such as the offshore migration of the spring thermal bar, currents, and other processes. Because of this offshore movement, long-term trends in inshore-region silicon concentrations may have affected long-term trends in the offshore silicon record as presented in Schelske and Stoermer.

This offshore movement of inshore water may also have affected the offshore paleolimnological record. Some inshore-region diatoms are transported offshore with water movements and eventually sediment to the lake floor. Other inshore-region diatoms settle to the lake floor (as intact or as grazed cells) and are then transported offshore with the movement of fine sediments. The strong presence of benthic diatoms in cores collected in the deepest region of Lake Michigan (Stoermer et al. 1990) is strongly suggestive of significant offshore transport of inshore-region diatoms.

In summary, Schelske and Stoermer have developed an interesting hypothesis regarding silicon depletion in Lake Michigan during the late 1950s and 1960s. The general validity of their top-down silicon depletion hypothesis can only be verified through experimental studies which consider trophic status and a variety of native and exotic planktivorous fish. The specific testing of their hypothesis will be difficult because of major limitations in sufficient quantitative data for this period. Paleolimnological studies appear to provide the best approach for investigating the dynamic period of the 1950s and 1960s — M.S. Evans, *Environment Canada, National Hydrology Research Institute, 11 Innovation Boulevard, Saskatoon, SK S7N 3H5, Canada.* (J12398)

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The First World Fisheries Congress was held in Athens, Greece, in May 1992 and more than 45 scientific and professional fisheries organisations worldwide gathered to consider the state of the world's fisheries. This Second Congress is a further opportunity for scientists, managers, and commercial and recreational industry to consider world developments in fisheries to learn of innovations and technological advances and consider where international science and management of our fisheries resources is going.

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